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(71) Applicant *(for all designated States except US)*: **DALHOUSIE UNIVERSITY** [CA/CA]; Office of the President, Arts and Administration Building, 6299 South Street, Halifax, Nova Scotia B3H 4H6 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DARVESH, Sultan** [CA/CA]; 6191 Duncan Street, Halifax, Nova Scotia B3L 1K1 (CA). **MAGEE, David, Ian** [CA/CA]; 228 Serenity Lane, Fredericton, New Brunswick E3B 7T3 (CA). **MCDONALD, Robert, S.** [CA/CA]; 27 Warwick Lane, Halifax, Nova Scotia B3M 4J3 (CA). **MARTIN, Earl, V.** [CA/CA]; 1611 Henry Street, Halifax, Nova Scotia B3H 3K2 (CA).

(74) Agents: **KOENIG, Hans** et al.; Smart & Biggar, P.O. Box 2999, Station D, 900 - 55 Metcalfe Street, Ottawa, Ontario K1P 5Y6 (CA).

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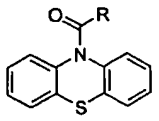
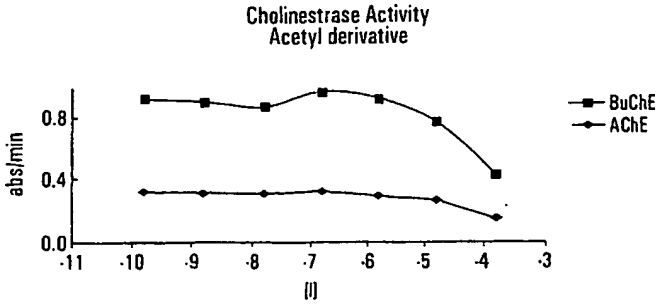
(54) Title: NOVEL N-SUBSTITUTED PHENOTHIAZINES AND THEIR USE AS MODULATORS OF SERINE HYDROLASE ENZYMES



(I)



(57) Abstract: The present invention is directed to phenothiazine compounds of formula (I), wherein R is: (a) a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by phenyl, halo or -NR_1R_2, wherein R_1 and R_2 are independently H, a branched or straight chain (C_1-C_6)alkyl group or R_1 and R_2 together with the nitrogen atom to which they are bonded form a 5- or 6-membered ring; (b) phenyl; or (c) -NR_3R_4, wherein R_3 and R_4 are independently; (i) H, (ii) a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by (C_1-C_4)alkoxy, phenyl or -NR_5R_6, wherein R_5 and R_6 are independently H, a branched or straight chain (C_1-C_4)alkyl group, phenothiazine carbonyl or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a 5- or 6-membered ring; (v) a (C_3-C_6)cycloalkyl group; or (iv) R_3 and R_4 together with the nitrogen atom to which they are bonded form pyrrolidino, piperidino, morpholino, piperazino or 4-methylpiperazino, or a pharmacologically acceptable salt thereof, for use in the treatment of Alzheimer's disease and other conditions. Compounds of formula (I) modulate the activity of serine hydrolase enzymes, for example, they are cholinesterase inhibitors.

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NOVEL N-SUBSTITUTED PHENOTHIAZINES AND THEIR USE AS MODULATORS OF SERINE HYDROLASE ENZYMES

FIELD OF THE INVENTION

The present invention is directed to novel N-substituted phenothiazines and their use as modulators of serine hydrolase enzymes.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a common neurodegenerative disorder causing dementia. The incidence of AD increases with age (1). The prevalence of dementia rises from 3% at age 65 years to 47% after age 85 years (1). The population of the elderly continues to rise and hence incidence of AD is also expected to rise. The frequency of dementia doubles every 5 years after the age of 60 years. In the United States, the annual cost for AD is estimated to be in excess of $60 billion annually (2, 3). With the rise in numbers of elderly individuals, the prevalence of AD is also expected to rise with concomitant rise in the cost for AD. Development of drugs to delay the progression of AD as well as provide symptomatic treatment of this disorder is thus of paramount importance (1, 2, 3).

In AD there are three major microscopic features that are recognized as the hallmarks of the disease, namely neuritic plaques (NP), neurofibrillary tangles (NFT) and amyloid angiopathy (AA) (4). In addition, there is

widespread cell loss, particularly of cholinergic neurons in the brain (5). Loss of cholinergic cells leads to reduction in the levels of the neurotransmitter acetylcholine, its synthesizing enzyme choline acetyltransferase, as well as its deactivating enzyme acetylcholinesterase (AChE) (5, 6). Reduction of cholinergic neurotransmission leads to some of the symptoms of AD (6).

Although the level of AChE is reduced in AD, the level of the closely related enzyme butyrylcholinesterase (BuChE 3.1.1.8) is increased in AD brains (7). BuChE is found in all the neuropathological lesions associated with AD, namely, NP, NFT and AA (7). Importantly, BuChE is found in NP in brains of patients with AD. BuChE is found in a higher number of plaques in brains of elderly individuals with AD relative to those without AD (8). BuChE in Alzheimer brains requires 10-100 times the concentration of inhibitors to completely inhibit its esterase activity relative to BuChE in normal brains (9). It has been shown that some BuChE inhibitors not only improve cognition in an animal model but also reduce the production of β-amyloid which is one of the principal constituents of neuritic plaques (10).

From a neuropathology perspective, deposition of amyloid and formation of NP is one of the central mechanisms in the evolution of AD (11, 12). However, amyloid plaques are also found in brains of elderly individuals who do not have dementia (13). It has been suggested that the amyloid plaques in individuals without dementia are "benign" and they become "malignant", causing

dementia, when they are transformed into plaques containing degenerated neurites (13). These plaques are called neuritic plaques (NP). The mechanism of transformation from "benign" to "malignant" plaques is as yet unknown. It has been suggested that BuChE may play a major role in this transformation based on the observation that BuChE is found predominately in plaques that contain dystrophic neurites and not in plaques without dystrophic neurites (13).

Taken together these observations suggest that in brains of patients with AD there is a significant alteration of the biochemical properties of BuChE that alters its normal regulatory role in the brain thus contributing to the pathology of AD.

Recently, a brain specific serine protease called trypsin IV has been isolated and it is presumed to be involved in APP processing (24). Amyloid precursor protein (APP) is a transmembrane glycoprotein, which possesses a Kunitz-type serine protease inhibitor domain. The APP may be involved in protease regulation in the brain (14, 15). Abnormally cleaved APP may result in the formation of a 40-42 amino acid residue ß-amyloid protein fragment. This fragment may be the main constituent of NP (16).

The proteolytic sites in APP have been studied extensively (18). There are three known proteolytic sites. The first is the α-secretase site which when cleaved yields a 120 KDa fragment that does not accumulate in amyloid plaques (18). A basic amino acid residue such as arginine at this site is required for cleavage (19). Enzymes that require a basic amino acid residue at the cleavage site of

their substrates are serine peptidases, such as trypsin. The second cleavage site, the γ-secretase site, cleaves at lys-28 (also a tryptic-site), which is the last amino acid of the extracellular APP domain (20). The third cleavage site, the ß-secretase site, occurs at the N-terminus (21). The latter two sites lead to fragments that accumulate in amyloid plaques.

The enzymes that cleave amyloid precursor protein are called "secretases" but they have not been fully identified (22). It has been observed that a basic amino acid residue is required at some of the sites where APP undergoes proteolytic cleavage (19). Two well-known enzymes that cleave peptides at basic amino acid residue sites are trypsin and carboxypeptidase B (23). Both of these enzymes are mainly recognized as pancreatic enzymes involved in digestion, but trypsin-like serine proteases have been found in the brain and are thought to be involved in APP processing (24, 25, 26, 27). Interestingly, an enzyme with tryptic-like activity is closely associated with BuChE (28, 29). Recent observations that BuChE considerably enhances tryptic activity under normal circumstances (30, 31) and the observations that BuChE, which is found in high levels in NP, has altered biochemical properties, suggests that there may be a loss of regulation of tryptic activity, and other serine peptidase activity, associated with BuChE. This loss of regulation may play a role in abnormal proteolytic processing of APP. Recent evidence suggests that inhibition of BuChE enhances cognitive performance in rats,

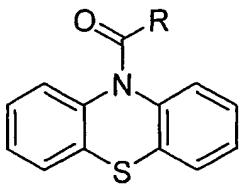
and that it promotes non-amyloidogenic processing of amyloid precursor protein (10).

Development of molecules that inhibit the activity of BuChE and/or AChE and simultaneously enhance the activity of serine proteases would not only provide symptomatic treatment of AD but would also lead to discovery of drugs that stop the progression of AD.

SUMMARY OF THE INVENTION

The present invention provides novel N-substituted phenothiazines, or pharmacologically acceptable salts thereof, that modulate serine hydrolase activity.

In accordance with the present invention, there is provided a compound of the formula (I):



(I)

wherein R is:

(a) a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by phenyl, halo or $-NR_1R_2$, wherein R_1 and R_2 are independently H, a branched or straight chain (C_1-C_6)alkyl group or R_1 and R_2 together with the nitrogen atom to which they are bonded form a 5- or 6-membered ring;

(b) phenyl; or

(c) $-NR_3R_4$, wherein R_3 and R_4 are independently;

(i) H,

(ii) a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by (C_1-C_4)alkoxy, phenyl or $-NR_5R_6$, wherein R_5 and R_6 are independently H, a branched or straight chain (C_1-C_4)alkyl group, phenothiazine-10-carbonyl or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a 5- or 6-membered ring,

(iii) a (C_5-C_6)cycloalkyl group, or

(iv) R_3 and R_4 together with the nitrogen atom to which they are bonded form pyrrolidino, piperidino, morpholino, piperazino or 4-methylpiperazino,

or a pharmacologically acceptable salt thereof.

The phenothiazines of the present invention, or pharmacologically acceptable salts thereof, inhibit the activity of cholinesterases, such as BuChE and AChE, and are useful in the treatment of Alzheimer's disease and/or other neurological disorders.

DETAILED DESCRIPTION

Preferably, R is a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by phenyl, or R is $-NR_3R_4$. More preferably, R is $-NR_3R_4$, a straight chain (C_1-C_4)alkyl group or a straight chain (C_1-C_4)alkyl group substituted by phenyl.

Preferably, R_3 and R_4 are independently H or a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by $-NR_5R_6$. More preferably, one of R_3 or R_4 is H and the other is a branched or straight chain (C_1-C_4)alkyl group substituted by $-NR_5R_6$.

Preferably, R_5 and R_6 are independently H, a branched or straight chain (C_1-C_4)alkyl group or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a saturated 5- or 6-membered ring. More preferably, R_5 and R_6 are independently a branched or straight chain (C_1-C_4)alkyl group or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a saturated 5- or 6-membered ring.

Most preferably, R is methyl, ethyl, n-propyl, $-CH_2$-phenyl, $-(CH_2)_2$-phenyl, $-NH-(CH_2)_2-NR_5R_6$ or $-NH-CH_2-C(CH_3)_2-CH_2-R_5R_6$, wherein R_5 and R_6 are methyl, ethyl or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a pyrrolidino or a piperidinyl ring.

The present invention extends to a pharmaceutical composition that comprises a phenothiazine of formula (I) as defined herein, or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically

acceptable diluents, carriers or excipients, for modulating serine hydrolase activity in a mammal, preferably a human. The pharmaceutical composition can be used to treat, inhibit or prevent a pathological condition that is manifested in an abnormal concentration of, and/or activity of, a serine hydrolase enzyme. Among those pathological conditions are Alzheimer's disease; tumours such as brain tumours, for example gliomas; glaucoma; cardiac disease; central nervous system disorders; respiratory infections; gastrointestinal diseases; renal diseases; and other dementias such as Lewy body dementia and vascular dementia.

Cholinesterases are not only involved in cholinergic neurotransmission but also in other biological processes such as development of the nervous system (33, 34). BuChE is found in high levels during neuroblast proliferation while AChE is found in high levels during neuronal maturation (34). BuChE is found in high levels in certain tumours, particularly primary brain tumour such as gliomas. Because BuChE is involved in the process of cellular proliferation, the phenothiazine compounds of the present invention that are more specific as BuChE inhibitors can be used to slow or stop growth of such brain tumours.

Glaucoma is one the common eye disease leading to blindness. In glaucoma, there is increased intraocular pressure. Intraocular pressure can be decreased by pupillary constriction. The pupil is innervated by both sympathetic (adrenergic) and parasympathetic (cholinergic) nervous systems. The parasympathetic nervous system, and cholinergic enhancing drugs, causes pupillary constriction,

which can reduce intraocular pressure. The phenothiazine compounds of the present invention that inhibit cholinesterases and raise acetylcholine levels could be used for the treatment of ophthalmic diseases such as glaucoma.

Thus, the active compounds of the invention may be formulated for oral, buccal, transdermal (for example, patch), intranasal, parenteral (for example, intravenous, intramuscular or subcutaneous), ophthalmic or rectal administration or in a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); filters (for example, lactose, microcrystalline cellulose or calcium phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycollate); or wetting agents (for example, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (for example, sorbitol syrup, methyl cellulose or

hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters or ethyl alcohol); and preservatives (for example, methyl or propyl p-hydroxybenzoates or sorbic acid).

For buccal administration the composition may take the form of tablets of lozenges formulated in conventional manner.

The active compounds of the invention may be formulated for parenteral administration by injection, including using conventional catheterization techniques or infusion. The active compounds of the invention may also be formulated for topical ophthalmic administration.

Formulations for injection or topical ophthalmic administration may be presented in unit dosage form, for example in ampoules, or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for reconstitution with a suitable vehicle, for example sterile pyrogen-free water, before use.

The active compounds of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, for example containing conventional suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, the active compounds of the invention are conveniently delivered in the form of a solution or suspension from a pump spray container that is squeezed or pumped by the patient. The compounds of the invention can also be delivered in the form of an aerosol spray presentation from a pressurized container or a nebulizer, with the use of a suitable propellant, for example dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

As used herein, the term "effective amount" means an amount of a compound of the invention that is capable of inhibiting the symptoms of a pathological condition described herein by modulation of serine hydrolase activity. The specific dose of a compound administered according to this invention will be determined by the particular circumstances surrounding the case including, for example, the compound administered, the route of administration, the state of being of the patient, and the severity of the pathological condition. A proposed dose of an active compound of the invention for oral, parenteral, buccal or topical ophthalmic administration to the average

adult human for the treatment of the conditions referred to above is 0.01 to 50 mg/kg of the active ingredient per unit dose which could be administered, for example, 1 to 4 times per day.

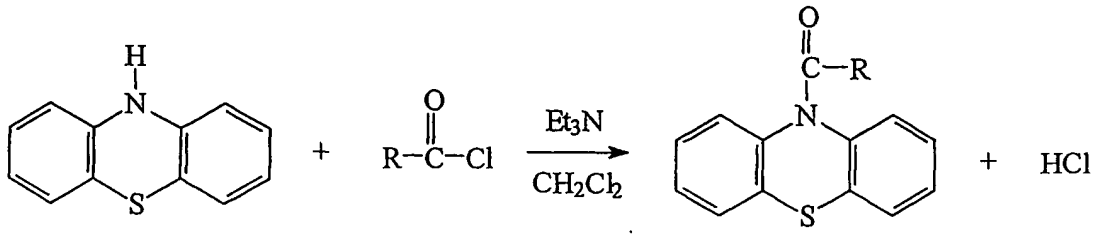
Aerosol formulations for treatment of the conditions referred to above in the average adult human are preferably arranged so that each metered dose or "puff" of aerosol contains 20μg to 1000μg of the compound of the invention. The overall daily dose with an aerosol will be within the range 100μg to 10 mg. Administration may be several times daily, for example 2, 3, 4 or 8 times, giving for example, 1, 2 or 3 doses each time.

The present invention also extends to the use and to methods of using the compounds and compositions described herein for treating the various conditions. The present invention also extends to the use of the compounds described herein for preparing a medicament for treating the various conditions.

The compounds and compositions are generally sold in the form of commercial packages or kits together with instructions for their use in treating the conditions described herein.

Synthetic approaches:

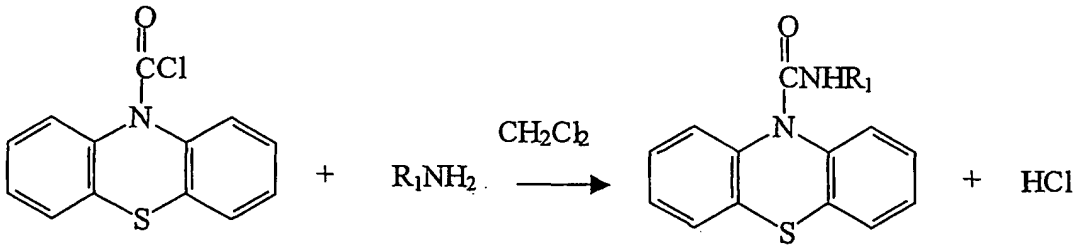
Scheme 1:



phenothiazine an amide

Scheme 2:

Scheme 2 involves the reaction of phenothiazine-10-carbonyl chloride (PT-10-COCl) with primary and secondary amines. Scheme 2 shows the general reaction with a primary amine to give an N-substituted phenothiazine urea. The reactions are generally fast, producing clean, easily-purified products.

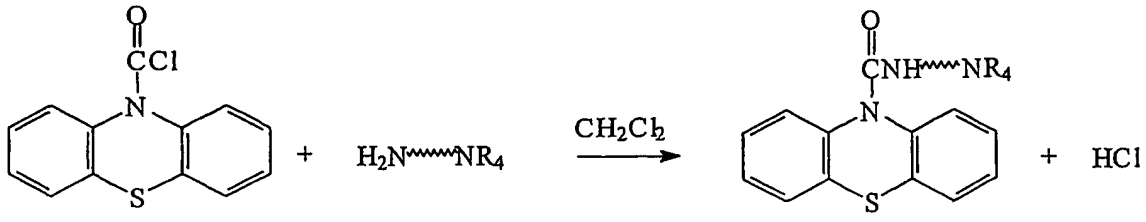


PT-10-COCl a urea

Scheme 3:

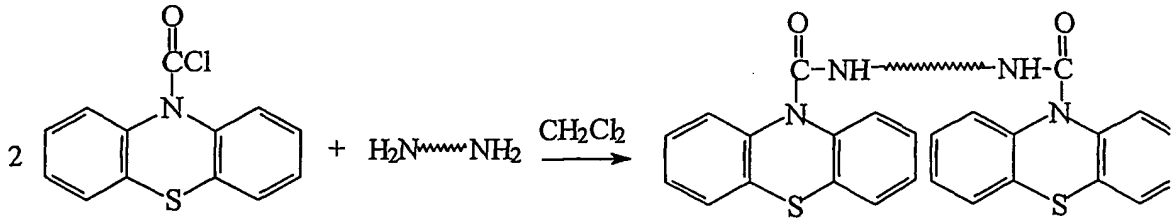
Compounds resulting from a reaction in accordance with Scheme 3 have a urea-type group (-N-(C=O)-N-), and, an amine functionality at the end of the N-substituted chain.

The linkage between the two nitrogen atoms in the chain can be varied using selected diamines.



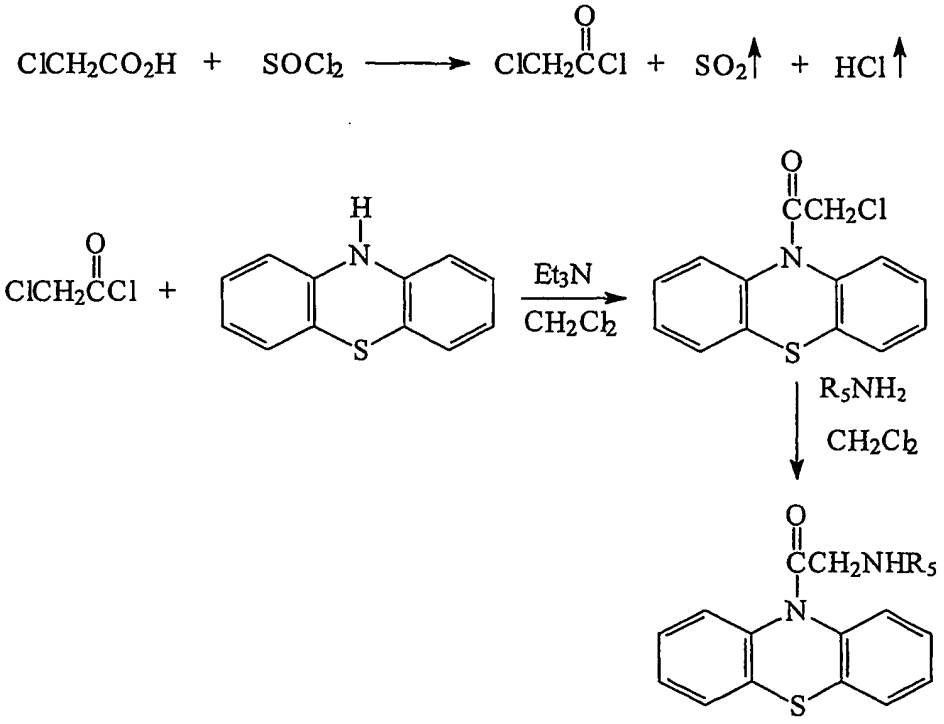
Scheme 4:

Diamines with two primary amino groups can form both the 1:1 product (as in Scheme 3) and the 2:1 product (as in Scheme 4) with PT-10-COCl. Adding the diamine dropwise to an excess of PT-10-COCl produced the 2:1 product. Reversing the order and adding the PT-10-COCl to an excess of diamine produced the corresponding 1:1 product.



Scheme 5:

Scheme 5 involves the acylation of the secondary amine functionality of phenothiazine with chloroacetyl chloride (prepared from thionyl chloride and chloroacetic acid) to produce N-chloroacetyl phenothiazine. N-chloroacetyl phenothiazine is then reacted with a variety of amines to produce an N-substituted phenothiazine with a methylene group between the amide carbonyl and the amine nitrogen.



Selected compounds:

Table 1 provides a list of selected compounds or salts that are within the scope of the invention.

TABLE 1

Cmpd.	Structure of R
1	$-CH_3$
2	$-CH_2CH_2CH_3$
3	$-CH_2$-Phenyl
4	$-CH_2CH_2$-Phenyl
5	$-NH-CH_2CH_2-N(CH_3)_2$
6	$-NH-CH_2CH_2-N(CH_2CH_3)_2$
7	$-NH-CH_2CH_2-NCH_2CH_2CH_2CH_2$
8	$-NH-CH_2CH_2-NCH_2CH_2CH_2CH_2CH_2$
9	$-NHCH_2C(CH_3)_2CH_2N(CH_3)_2$
10	$-NH-CH_2CH_2-NH_2$
11	$-NH-CH_2CH_2CH_2-NH_2$
12	$-NH-CH_2C(CH_3)_2CH_2-NH_2$
13	$-NH-CH_2CH_2-N^+H(CH_3)_2$
14	$-NH-CH_2CH_2-N^+H(CH_2CH_3)_2$

TABLE 1 - continued

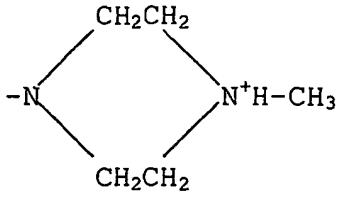
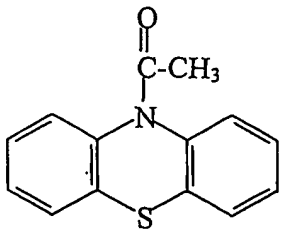
Cmpd.	Structure of R
15	$-NH-CH_2CH_2CH_2-N(CH_3)_2$
16	$-NH-CH_2CH_2CH_2-N^+H(CH_2CH_3)_2$
17	$-NH-CH_2C(CH_3)_2CH_2-N(CH_3)_2$
18	
19	$-NH-CH_2CH_2-NH-(PT-10-CO)^1$
20	$-NH-CH_2CH_2CH_2-NH-(PT-10-CO)^1$
21	$-NH-CH_2C(CH_3)_2CH_2-NH-(PT-10-CO)^1$
22	$-CH_2Cl$
23	$-CH_2-N^+H_2-CH_2CH_2CH_3$
24	$-CH_2-N^+H_2-CH_2CH_2CH_2CH_3$
25	$-CH_2-N^+H_2-CH_2CH(CH_3)_2$
26	$-CH_2$-Pyrrolidino
27	$-CH_2CH_3$
28	$-CH_2CH(CH_3)_2$
29	-Phenyl

TABLE 1 - continued

Cmpd.	Structure of R
30	$-NH-CH_2CH_2CH_3$
31	$-NH-CH_2CH_2CH_2CH_3$
32	$-NH-CH_2CH(CH_3)_2$
33	$-NH-CH(CH_3)(CH_2CH_3)$
34	$-NH-C(CH_3)_3$
35	$-NH-CH_2CH_2OCH_3$
36	$-NH-CH_2C(CH_3)_3$
37	-NH-Cyclohexyl
38	$-NH-CH_2$-Phenyl
39	$-N(CH_2CH_3)_2$
40	-(1-Pyrrolidino)
41	-(1-Piperidino)
42	-Morpholino
43	$-NH-CH_2CH_2CH_2-N(CH_2CH_3)_2$

[1] PT-10-CO is a phenothiazine-10-carbonyl radical bonded to the rest of the molecule through the carbonyl carbon atom.

Compounds 1 to 26 have the formulae as shown below:



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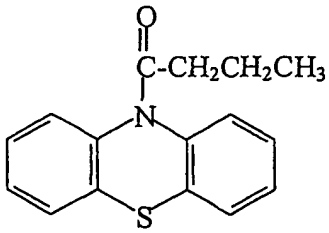

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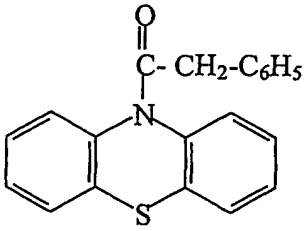

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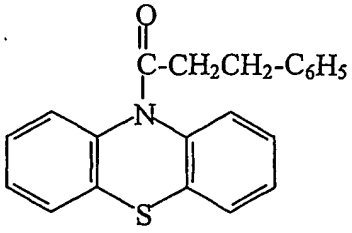

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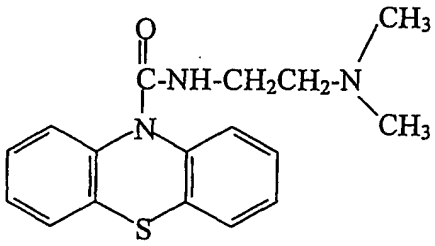

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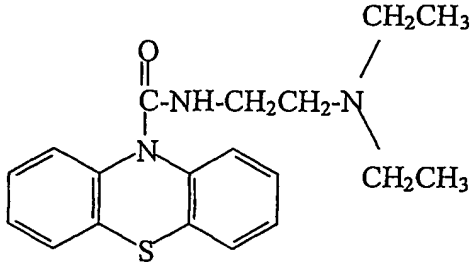

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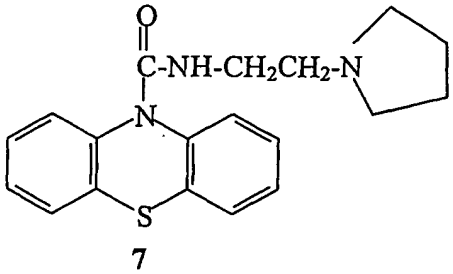

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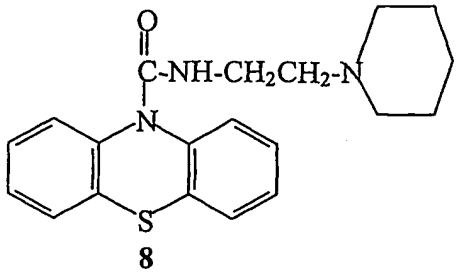

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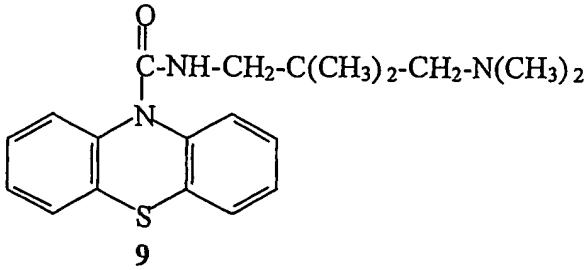

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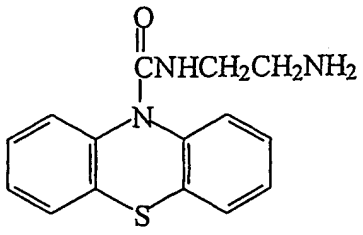

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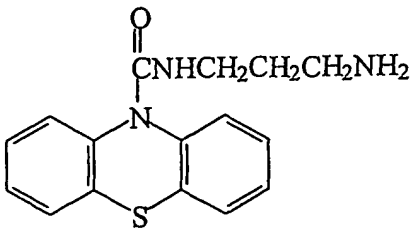

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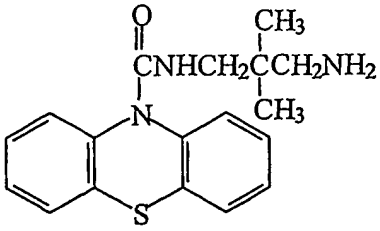

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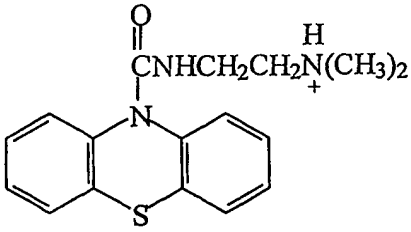

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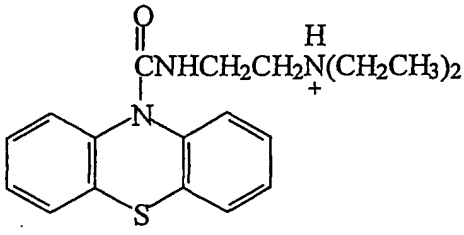

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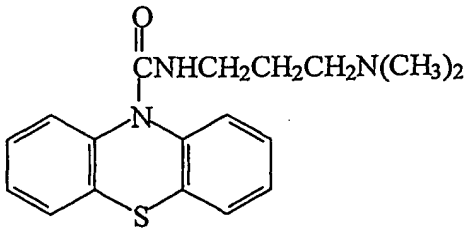

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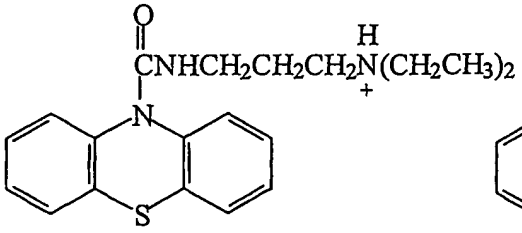

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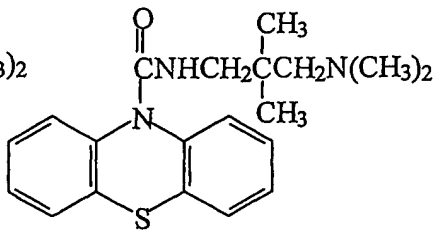

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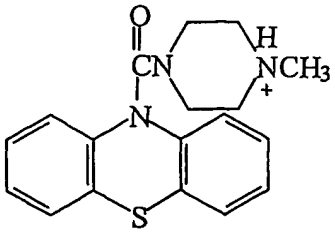

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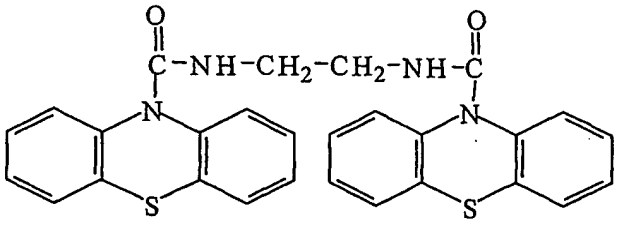

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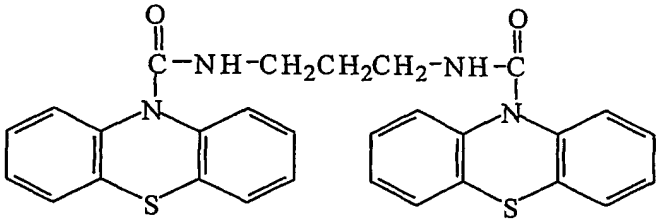

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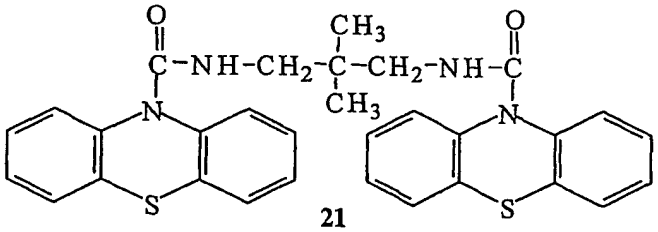

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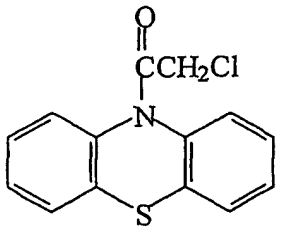

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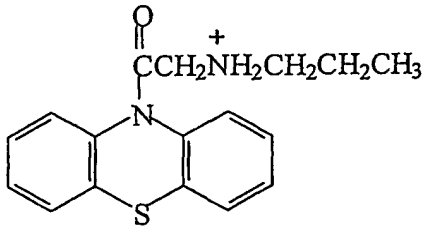

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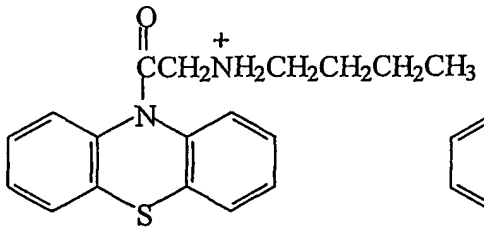

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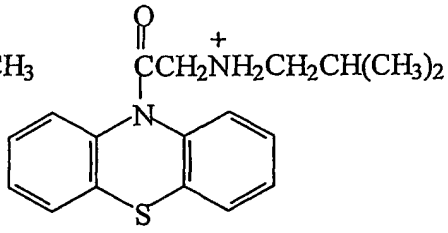

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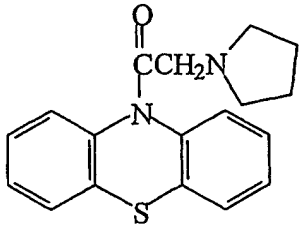

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SYNTHETIC EXAMPLES

General Analytical Methods:

Melting points were recorded on a Mel-Temp™ II apparatus and are uncorrected. Infrared spectra were recorded as Nujol™ mulls on sodium chloride plates using a Nicolet™ Model 205 FT-IR spectrometer. Peak positions were obtained in "Peak Pick" mode. The nuclear magnetic resonance (NMR) spectra were determined on a Bruker™ AC250F

spectrometer at The Atlantic Region Magnetic Resonance Centre. This instrument operates at 250 MHz for proton NMR and 62.9 MHz for carbon. Chemical shifts are reported in ppm relative to TMS. Mass spectra were recorded on a CEC 21-110B mass spectrometer at Dalhousie University or on a Kratos™ MS50 mass spectrometer at the University of New Brunswick.

Phenothiazine and phenothiazine-10-carbonyl chloride (PT-10-COCl) were purchased from Aldrich™ and Acros™, respectively, and used without further purification. The amines were purified by fractional or simple distillation. All reactions were performed under anhydrous conditions. The reactions were monitored by TLC using plastic-backed silica plates with fluorescent indicator and CH_2Cl_2 as developing solvent. Phenothiazine and phenothiazine-10-carbonyl chloride both have R_f values of ~0.61 under these conditions while the products remain close to the origin. Although the compounds were homogeneous as indicated by TLC, some of the ^1H NMR spectra showed small amounts of impurities.

General Synthesis of Amides:

To a stirred solution of 5.1 mmol phenothiazine and 5.1 mmol triethylamine in 20 mL dichloromethane are added dropwise a solution containing 12.5-25 mmol of the desired acyl chloride (R-COCl) in 5 mL dichloromethane. The reaction mixture is then refluxed until all phenothiazine is consumed as judged by silica gel thin layer chromatography using dichloromethane as eluting

solvent. The cooled reaction mixture is then washed successively with 4 x 30 mL of 5% sodium bicarbonate, then 3 x 30 mL 5% hydrochloric acid and finally with water. The organic layer is then dried over magnesium sulphate, filtered, and the solvent evaporated. The crude solid product is then purified by recrystallization from petroleum ether-dichloromethane (2:1), with or without prior column chromatography. Yields range from 9-50%.

General Synthesis of Ureas:

To a stirred solution of 3.86 mmol phenothiazine-10-carbonyl chloride in 20 mL dichloromethane is added dropwise a solution containing 9.5-11.5 mmol of the desired amine dissolved in 5 mL dichloromethane. After stirring for one hour at room temperature, thin layer chromatography generally reveals that all of the 10-carbonyl chloride is consumed.

The reaction mixture for Compound 6 produced a precipitate at this point. It was stirred for a further 24 hours at which time the precipitate was removed by filtration, washed with dichloromethane and allowed to air-dry. This solid proved to be the desired Compound 6 in the form of the hydrochloride salt.

Other urea reaction mixtures were subjected to the following work-up procedure. The organic solution was washed successively with 4 x 30 mL portions of 0.1 M sodium hydroxide, once with a 30 mL portion of 0.1 M hydrochloric acid, twice with 30 mL portions of 0.1 M sodium hydroxide,

and finally with water. The solution was dried (magnesium sulphate), filtered and the solvent evaporated.

The crude products for Compounds 7, 8 and 9, for example, did not crystallize at this point and were converted directly into the hydrochlorides by taking up the reaction mixture in 7-10 mL diethyl ether, followed by the dropwise addition of 5-7 drops of concentrated hydrochloric acid. The precipitated salts were then removed by filtration, washed with ether, and allowed to air-dry.

Product yields vary from 11-60%.

Synthesis of Particular Compounds:

Compounds 10-12. PT-10-COCl (1 g, 4 mmol) was dissolved in CH_2Cl_2 (20 mL) and this solution was slowly added through a dropping funnel (over the period of an hour) to a well-stirred solution of the diamine (12 mmol) in CH_2Cl_2 (15 mL). The reaction was essentially complete within 5-10 minutes after addition of the PT-10-COCl solution as monitored by TLC. Any precipitate in the reaction mixture was gravity filtered, characterized by IR and was determined to be either the 2:1 product or the hydrochloride salt of the diamine or a mixture of both. The CH_2Cl_2 solution was extracted with 0.1 N NaOH (2 × 30 mL), washed with distilled H_2O (2 × 30 mL), dried with $MgSO_4$, filtered and evaporated to dryness on a rotary evaporator.

Compound 10. By the procedure above, reaction between PT-10-COCl (1.00 g, 3.9 mmol) and 1,2-ethanediamine (0.69 g, 12 mmol) gave the compound as an oily residue. On

cooling to -20°C, white crystals formed (0.71g as free base, 54%). Characterization by ^1H NMR indicated that the compound contained slight impurities. Recrystallization from CH_2Cl_2/pentane failed to remove these impurities.

Compound 11. This compound was prepared from PT-10-COCl (0.97 g, 3.7 mmol) and 1,3-propanediamine (0.94 g, 12.7 mmol) according to the procedure above. The CH_2Cl_2 solution was extracted with 0.1 N NaOH and then 0.1 N HCl. The aqueous acid layers were combined and made basic by addition of NaOH pellets (20 pellets were required); the solution turned milky white. The basic solution was extracted with CH_2Cl_2 (2 × 30 mL). The organic layers were combined, dried with $MgSO_4$, gravity filtered and evaporated to dryness on a rotary evaporator to give a gum. Addition of diethyl ether (10 mL) induced the formation of white crystals. Evaporation of the solution yielded the compound (0.82 g as free base, 72%).

Compound 12. By the procedure above, reaction between PT-10-COCl (1.00 g, 3.8 mmol) and 2,2-dimethyl-1,3-propanediamine (1.17 g, 11.5 mmol) yielded compound 12 as a pale orange, sticky solid. The solid was recrystallized from H_2O/MeOH and air-dried to give the compound as a white powder (0.21 g as free base, 17 %).

Compound 13. N,N-dimethyl-1,2-ethanediamine (0.33 g, 3.9 mmol) was added dropwise to PT-10-COCl (1.02 g, 3.9 mmol) in CH_2Cl_2 (25 mL) with stirring. A white precipitate had formed after 24 hours of stirring. The precipitate was filtered and rinsed with CH_2Cl_2 to give the compound (0.62 g as HCl salt, 47%).

Compounds 14-18. The diamine (9-12 mmol) in CH_2Cl_2 (5 mL) was added through a dropping funnel to a solution of PT-10-COCl (1.00 g, 3.9 mmol) in CH_2Cl_2 (25 mL) with stirring. The reaction was complete after an additional 5-10 minutes of stirring as monitored by TLC. The CH_2Cl_2 solution was extracted with 0.1 N NaOH (2 × 50 mL), washed with distilled H_2O, dried with $MgSO_4$, filtered and evaporated to dryness on a rotary evaporator. If the product smelled of amine, it was dissolved in CH_2Cl_2 (25 mL) and extracted with 0.1 N HCl (2 x 30 mL) and then with 0.1 N NaOH (2 x 30 mL). The CH_2Cl_2 layer was washed with distilled H_2O, dried with $MgSO_4$, filtered and evaporated to dryness on the rotary evaporator. If an oil resulted, it was taken up in diethyl ether (10 mL) and concentrated HCl was added dropwise (5-6 drops were required) to precipitate the product as the hydrochloride salt. The solution was gravity filtered and the product dried in a desiccator.

Compound 14. By the procedure above, reaction of PT-10-COCl (1.02 g, 3.9 mmol) with N,N-diethyl-1,2-ethanediamine yielded the compound (0.58 g as HCl salt, 45%).

Compound 15. By the procedure above, reaction of PT-10-COCl (1.01 g, 3.9 mmol) with N,N-dimethyl-1,3 propanediamine (0.97 g, 9.5 mmol) yielded the compound as a white powder (0.78 g as free base, 63%).

Compound 16. By the procedure above, reaction between PT-10-COCl (1.00 g, 3.8 mmol) and N,N-diethyl-1,3-propanediamine (1.25 g, 9.6 mmol) yielded a yellow oil.

Conversion of the product to the hydrochloride salt gave the compound as a white powder (0.54 g as HCl salt, 36%).

Compound 17. By the procedure above, reaction between PT-10-COCl (1.00 g, 3.8 mmol) and N,N,2,2-tetramethyl-1,3-propanediamine (1.44 g, 11.1 mmol) gave the compound as a white powder (0.14 g as free base, 10%).

Compound 18. By the procedure above, reaction between PT-10-COCl (1.00 g, 3.8 mmol) and 1-methylpiperazine (1.43 g, 14.3 mmol) gave the compound as a white powder (0.36 g as HCl salt, 26%).

Compounds 19-20. The diamine (4 mmol) was added through a dropping funnel to a well-stirred solution of PT-10-COCl (1.00 g, 3.9 mmol). A voluminous white precipitate formed, which was filtered, rinsed with CH_2Cl_2, air-dried and characterized.

Compound 19. By the procedure above, reaction between PT-10-COCl (1.03 g, 3.9 mmol) and 1,2-ethanediamine (0.23 g, 3.8 mmol) gave the compound as a white powder (1.00 g, 100%).

Compound 20. By the procedure above, reaction between PT-10-COCl (1.00 g, 3.8 mmol) and 1,3-propanediamine yielded the compound as a white powder (0.93 g, 93%).

Compound 21. 2,2-dimethyl-1,3-diaminopropane (0.24 g, 2.3 mmol) in CH_2Cl_2 (5 mL) was added through a dropping funnel to a solution of PT-10-COCl (1.00 g, 3.9 mmol) in CH_2Cl_2 (20

mL) with stirring. After 48 hours of stirring, starting material was still present as indicated by TLC. A white precipitate had formed and was gravity filtered. From the IR spectrum, the precipitate was determined to be the hydrochloride salt of 2,2-dimethyl-1,3-diaminopropane. The reaction mixture was evaporated to dryness on a rotary evaporator to give a white solid (0.62 g). TLC of the solid in CH_2Cl_2 showed unreacted PT-10-COCl (R_f = 0.60) and the presumed 2:1 product (at origin). With 5% MeOH/CH_2Cl_2 as the developing solvent, both spots moved: R_f=0.82 for PT-10-COCl and R_f=0.69 for the presumed product. Based on the TLC results, the solid was subjected to column chromatography using 20 g of silica gel; the PT-10-COCl was eluted with CH_2Cl_2. On elution with 5% MeOH/CH_2Cl_2, the compound was isolated as a pale pink powder (0.29 g, 23%).

Compound 22. (10-chloroacetylphenothiazine, PT-10-$COCH_2Cl$). Chloroacetyl chloride was prepared by adding thionyl chloride (32 mL, 0.44 mol) through a dropping funnel to chloroacetic acid (50 g, 0.53 mol). The reaction mixture was refluxed for two hours and then distilled using a fractionation column (bp_{obs}=105.0-105.5, bp_{lit}=105). Only 8 mL of chloroacetyl chloride were collected (10.35 g, 0.092 mol). Phenothiazine (10.00 g, 50 mmol) was dissolved in 200 mL CH_2Cl_2 and triethylamine (5.00 g, 50 mmol) was added to the solution. Chloroacetyl chloride (10.35 g, 92 mmol) in CH_2Cl_2 (10 mL) was added through a dropping funnel to the solution. The reaction mixture was refluxed for 24 hours and was monitored by TLC. Disappearance of the PT spot (R_f=0.61) indicated that the reaction was complete. The product had an R_f value of 0.32 in CH_2Cl_2. The reaction

mixture was extracted with 5% $NaHCO_3$ (3 x 50 mL), 5% HCl (3 x 50 mL) and then 5% $Na_2S_2O_3$ (3 x 50 mL). The CH_2Cl_2 layer was washed with distilled H_2O (50 mL), dried with $MgSO_4$, gravity filtered and evaporated to dryness to give 8.92 g (65%) of the crude product. Recrystallization afforded off-white crystals, which appeared to be homogeneous by TLC and NMR.

Compounds 23-26. The amine (5 mmol) in CH_2Cl_2 was added through a dropping funnel to a solution of PT-10-$COCH_2Cl$ (0.50 g, 1.9 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was refluxed and monitored by TLC frequently. When the reaction was complete, as judged by disappearance of the spot at R_f=0.32, the CH_2Cl_2 solution was extracted with 0.1 N NaOH (3 x 30 mL), washed with distilled H_2O (30 mL), dried with $MgSO_4$, gravity filtered and evaporated to dryness on a rotary evaporator. If a solid resulted, it was recrystallized from petroleum ether/CH_2Cl_2. If an oil resulted, it was taken up in diethylether (10 mL) and concentrated HCl was added (4-6 drops were required) to convert the amine product to the HCl salt, which precipitated from the solution. The solution was gravity filtered and the solid was dried in a desiccator.

Compound 23. By the procedure above, reaction between PT-10-$COCH_2Cl$ (0.39 g, 1.4 mmol) and n-propylamine (0.21 g, 3.5 mmol) gave the compound as a pink solid (77 mg as HCl salt, 16%). The reaction was complete after refluxing the reaction mixture for 5 hours and stirring for two days.

Compound 24. By the procedure above, reaction of PT-10-$COCH_2Cl$ (0.51 g, 1.9 mmol) and n-butylamine (0.41 g, 5.6 mmol) gave the compound as a white powder (60 mg as HCl salt, 9%). The reaction was complete after 20 hours of refluxing.

Compound 25. By the procedure above, reaction between PT-10-$COCH_2Cl$ (0.48 g, 1.7 mmol) and isobutylamine (0.38 g, 5.22 mmol) gave the compound as a white powder (62 mg as HCl salt, 10%). The reaction was complete after refluxing the reaction mixture for 4 hours and stirring for 2 days.

Compound 26. This compound was prepared from PT-10-$COCH_2Cl$ (0.53 g, 1.9 mmol) and pyrrolidine (0.41 g, 5.7 mmol) according to the procedure above. The reaction was complete after 45 minutes of refluxing and the isolated product was recrystallized from petroleum ether/CH_2Cl_2 (0.34 g as free base, 60%).

Analytical Data for Compounds 1-9:

Compounds 1-9 were prepared by adapting the general syntheses of amides and ureas as described above.

Compound 1: 10-Acetyl-10*H*-phenothiazine

^1H NMR ($CDCl_3$): δ 2.19 (s, 3H), δ 7.2 (t, J=7.5 Hz; d, J=1.5 Hz, 2H), δ 7.31 (t, J=7.5 Hz; d, J=1.5 Hz, 2H), δ 7.42 (d, J=7.5 Hz; d J=1.5 Hz, 2H), δ 7.49 (d, J=7.5 Hz, 2H)

^{13}C NMR: 23.9, 127.7, 127.9, 128.1, 128.8, 139.8, 170.2

Infrared (IR) (Nujol™): 1671 cm^{-1}, 1321 cm^{-1}, 1259 cm^{-1}, 766 cm^{-1}

Mass Spectrum (MS): M^+ (observed) = 241.0567; calculated for $C_{14}H_{11}NOS$ = 241.0561

Melting Point (MP): 195-200^{0}C

Compound 2: 10-butyryl-10*H*-phenothiazine

^1H NMR ($CDCl_3$): δ 0.86 (t, J=7.3 Hz, 3H), δ 1.62 (m, J=7.3 Hz, 2H), 2.43 (t, J=7.3 Hz, 2H), δ 7.22 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.32 (t, J=7.6 Hz; J=1.5 Hz, 2H), δ 7.44 (d, J=7.6 Hz; d, J=1.5 Hz, 2H), δ7.50 (d, J=7.9 Hz; d, J=1.2 Hz, 2H)

IR (Nujol™): 1678 cm^{-1}, 1250 cm^{-1}, 1180 cm^{-1}, 765 cm^{-1}, 755 cm^{-1}

m/e 100%, 199.1; MS: M^+ (observed) = 269.0864; calculated for $C_{16}H_{15}ONS$ = 269.0874

MP: 82-84^{0}C

Compound 3: 10-(phenylacetyl)-10*H*-phenothiazine

^1H NMR ($CDCl_3$): δ 3.81 (s, 2 H), δ 7.05 - 7.53 (m, 13H)

IR (Nujol™): 1681 cm^{-1}, 1662 cm^{-1}, 1342 cm^{-1}, 770 cm^{-1}, 759 cm^{-1}

MS: M^+ (observed) 317.0877; calculated for $C_{20}H_{15}NOS$ = 317.0874

MP: 150-153.5^{0}C

Compound 4: 10-(3-phenylpropanoyl)-10*H*-phenothiazine

^{1}H NMR ($CDCl_3$): δ 2.75 (t, J=7.5 Hz, 2 H), δ 2.94 (t, J= 7.5 Hz, 2H); δ 7.07-7.46 (m, 13H)

^{13}C NMR: 31.5, 36.2, 126.2, 126.9, 127.0, 127.3, 128.0, 128.4, 128.5, 133.4, 138.8, 140.9, 171.4

IR (Nujol™): 1673 cm^{-1}, 1310 cm^{-1}, 1249 cm^{-1}, 767 cm^{-1}, 753 cm^{-1}, 693 cm^{-1}

MS: M^+ (observed) 331.1015; calculated for $C_{21}H_{17}NOS$ = 331.1031

MP: 102-104°C

Compound 5: N-[2-(dimethylamino)ethyl]-10H-phenothiazine-10-carboxamide

^{1}H NMR (DMSO-d_6): δ 2.76 (s, 6H), δ 3.14 (t, J=5.8 Hz, 2H), δ 3.43 (q, J=5.8 Hz, 2H), δ 6.82 (t, J=5.8 Hz, 1 H), δ 7.26 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.37 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.49 (d, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.68 (d, J=7.9 Hz; d, J=1.2 Hz, 2H), δ 10.68 (s, 1H)

IR (Nujol™): 3338 cm^{-1}, 2368 cm^{-1}, 1656 cm^{-1}, 766 cm^{-1}

MS: M^+ = 313.1260 (observed), calculated for $C_{17}H_{19}N_3OS$ = 313.1249

MP: 208-209°C

Compound 6: N-[2-(diethylamino)ethyl]-10H-phenothiazine-10-carboxamide

^{1}H NMR (DMSO-d_6): δ 1.20 (t, J=7.2 Hz, 6H), δ 3.08 (m, 6H), δ 3.43 (q, J=6.1 Hz, 2H), δ 6.84 (t, J=5.4 Hz; 1H), δ 7.25 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.36 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.47 (d, J=7.6 Hz; d, J=1.5 Hz, 2H), δ7.60 (d, J=7.9 Hz; d, J= 0.9 Hz, 2H), δ 10.72 (s, 1H)

IR (Nujol™): 3366 cm^{-1}, 2600 cm^{-1}, 2430 cm^{-1}, 1658 cm^{-1}, 1512 cm^{-1}, 771 cm^{-1}

MS: M^+ 342 (observed), calculated for $C_{19}H_{23}N_3OS$ = 341

MP: 184-186^{0}C

Compound 7: N-(2-pyrrolidin-1-ylethyl)-10H-phenothiazine-10-carboxamide

^{1}H NMR ($CDCl_3$): δ 1.78 (very broad s, 4 H), δ 2.07 (broad s, 4H), δ 3.27 (t, J=6.3Hz, 2H), δ 3.73 (q, 6.1 Hz; 2H), δ 6.13 (t, J=5.6 Hz, 1H), δ 7.24 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.37 (t, J=7.6 Hz, d, J=1.5 Hz, 2H), δ 7.43 (d, J=7.6 Hz; d, J=1.5 Hz, 2H), δ7.57 (d, J=7.6 Hz; d, J=1.5 Hz, 2H)

^{13}C NMR ($CDCl_3$): δ 23.2, 37.4, 54.5, 54.7, 126.9, 127.2, 127.5, 128.0, 133.3, 138.1, 155.2

IR (Nujol™): 3376 cm^{-1}, 2700cm^{-1}, 2630 cm^{-1}. 2493 cm^{-1}, 1667 cm^{-1}, 1503 cm^{-1}, 769 cm^{-1}, 756 cm^{-1}

MS: M^+ (observed), calculated for $C_{19}H_{21}N_3OS$ = 339

MP: 189-191^{0}C

Compound 8: N-(2-piperidin-1-ylethyl)-10H-phenothiazine-10-carboxamide

^{1}H NMR ($CDCl_3$): δ 1.56 (broad s, 2H), δ1.91 (broad s, 4H), δ 3.0-3.1 (broad s, 3H), δ 3.03 (t, J=6.3 Hz, 2H), δ 3.71 (q, J=6.1 Hz, 2H), δ 6.30 (t, J=5.7 Hz, 1 H), δ 7.21 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.34 (t, J=7.6 Hz; d, J=1.5 Hz, 2H), δ 7.39 (d, J=7.6 Hz; d, J=1.5 Hz, 2H), δ7.54 (d, J=7.9 Hz; d J=1.2 Hz, 2H)

^{13}C NMR ($CDCl_3$): δ 21.6, 22.7, 35.6, 52.4, 55.4, 126.5, 127.3, 127.5, 127.8, 132.2, 138.7, 154.2

IR (Nujol™): 3379 cm^{-1}, 2634 cm^{-1}, 2530 cm^{-1}, 1665 cm^{-1}, 1509 cm^{-1}, 1317 cm^{-1}, 1255 cm^{-1}, 769 cm^{-1}, 755 cm^{-1}

MS: M^+ (observed), calculated for $C_{20}H_{23}N_3OS$ = 353

MP: 122-140°C (decomposes)

Compound 9: N-[3-(dimethylamino)-2,2-dimethylpropyl]-10H-phenothiazine-10-carboxamide

^{1}H NMR ($CDCl_3$): δ 0.86 (s, 6H), δ 1.83 (s, 6H), δ 2.09 (s, 2H), δ 3.16 (d, J=4.3 Hz, 2H), δ 7.17 (t, J=7.5 Hz, d, J=1.3 Hz, 2H), δ 7.31 (t, J=7.7, d, J=1.6 Hz, 2H), δ 7.37 (d, J=7.6 Hz, d J=1.5 Hz, 2H), δ7.59 (d, J=7.9 Hz, d J=1.2 Hz, 2H), δ 7.84 (t broad, 1H)

IR (Nujol™): 3247 cm^{-1}, 1668 cm^{-1}, 1508 cm^{-1}, 1760 cm^{-1}

MS: M^+ 356 (observed), calculated for $C_{20}H_{25}N_3OS$ = 355

MP: 140-142.5°C

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 to 26 show inhibition of enzyme activity by compounds of the invention.

Figure 1 shows selectivity, mode and strength of inhibition displayed by acetyl PTZ (compound 1) towards BuChE and AChE.

Figure 2 shows selectivity, mode and strength of inhibition displayed by benzoyl PTZ (compound 29) towards BuChE.

Figure 3 shows selectivity, mode and strength of inhibition displayed by butanoyl PTZ (compound 2) towards BuChE and AChE.

Figure 4 shows selectivity, mode and strength of inhibition displayed by chloroacetyl PTZ (compound 22) towards BuChE and AChE.

Figure 5 shows selectivity, mode and strength of inhibition displayed by propanoyl PTZ (compound 27) towards BuChE and AChE.

Figure 6 shows selectivity, mode and strength of inhibition displayed by iso-valeryl PTZ (compound 28) towards BuChE.

Figure 7 shows selectivity, mode and strength of inhibition displayed by n-propyl urea PTZ (compound 30) towards BuChE.

Figure 8 shows selectivity, mode and strength of inhibition displayed by butyl urea PTZ (compound 31) towards BuChE.

Figure 9 shows selectivity, mode and strength of inhibition displayed by iso-butyl urea PTZ (compound 32) towards BuChE.

Figure 10 shows selectivity, mode and strength of inhibition displayed by sec-butyl urea PTZ (compound 33) towards BuChE.

Figure 11 shows selectivity, mode and strength of inhibition displayed by tert-butyl urea PTZ (compound 34) towards BuChE.

Figure 12 shows selectivity, mode and strength of inhibition displayed by 2-methoxyethyl urea PTZ (compound 35) towards BuChE.

Figure 13 shows selectivity, mode and strength of inhibition displayed by diethyl urea PTZ (compound 39) towards BuChE.

Figure 14 shows selectivity, mode and strength of inhibition displayed by neopentyl urea PTZ (compound 36) towards BuChE.

Figure 15 shows selectivity, mode and strength of inhibition displayed by pyrrolidine urea PTZ (compound 40) towards BuChE.

Figure 16 shows selectivity, mode and strength of inhibition displayed by piperidine urea PTZ (compound 41) towards BuChE.

Figure 17 shows selectivity, mode and strength of inhibition displayed by cyclohexyl urea PTZ (compound 37) towards BuChE.

Figure 18 shows selectivity, mode and strength of inhibition displayed by morpholine urea PTZ (compound 42) towards BuChE.

Figure 19 shows selectivity, mode and strength of inhibition displayed by N,N-dimethyl ethylene diamine urea PTZ (compound 5) towards BuChE and AChE.

Figure 20 shows selectivity, mode and strength of inhibition displayed by benzyl urea PTZ (compound 38) towards BuChE.

Figure 21 shows selectivity, mode and strength of inhibition displayed by ethylene diamine urea (monomer) PTZ (compound 10) towards BuChE.

Figure 22 shows selectivity, mode and strength of inhibition displayed by ethylene diamine urea (2:1 product) PTZ (compound 19) towards BuChE and AChE.

Figure 23 shows selectivity, mode and strength of inhibition displayed by N,N-diethyl ethylene diamine urea PTZ (compound 6) towards BuChE and AChE.

Figure 24 shows selectivity, mode and strength of inhibition displayed by N,N-dimethyl propylene diamine urea PTZ (compound 15) towards BuChE and AChE.

Figure 25 shows selectivity, mode and strength of inhibition displayed by N,N-diethyl propylene diamine urea PTZ (compound 43) towards BuChE and AChE.

Figure 26 shows selectivity, mode and strength of inhibition displayed by 1,3-propyl diamine urea PTZ (compound 11) towards AChE.

BIOCHEMICAL STUDIES

General Materials and Methods:

Preparation of Reagents and Enzymes:

A) 5,5'-Dithio-bis(2-Nitrobenzoic Acid) (DTNB) Stock

In 20 mL of 0.1 M phosphate buffer (pH 7.0), 0.03 g of sodium bicarbonate and 0.079 g of DTNB were combined and mixed.

B) Buffered 5,5'-Dithio-bis(2-Nitrobenzoic Acid) (DTNB) solution

3.6 mL of stock DTNB was combined with 96.4 mL of 0.1M phosphate buffer (pH 8.0).

C) Acetylthiocholine (AcTCH)

0.086 g of AcTCH was dissolved in 20 mL of distilled water to give a stock concentration of 15.0 mM. 0.1 mL of the stock solution in a final volume of 3.0 mL gave a concentration of 0.50 mM in the cuvette. A number of 50% serial dilutions were performed from the stock solution to produce the substrate concentrations employed in the assay.

D) Butylthiocholine (BuTCH)

0.0952g of BuTCH was dissolved in 20 mL of distilled water to give a concentration of 15.0 mM. 0.1 mL of the stock solution in a final volume of 3.0mL gave a concentration of 0.50 mM in the cuvette. A number of 50% serial dilutions were performed from the stock solution to produce the substrate concentrations employed in the assay.

E) Human Butylcholinesterase (BuChE)

1.0 mL of 0.005% aqueous gelatin was added to a stock bottle containing 100U of enzyme. Appropriate ratios of stock enzyme solution and 0.005% aqueous gelatin were combined, such that the diluted enzyme solution gave a change in absorbance per minute of approximately 1.00 at the highest concentration of BuTCH (i.e. 0.50 mM).

F) Human Acetylcholinesterase (AChE)

0.0206g of enzyme was combined with 4.0 mL of either 0.005% aqueous gelatin or 0.5% Triton-X 100, and ground to a slurry with a mortar and pestle. The resulting enzyme solution gave a change in absorbance per minute of

approximately 0.300 at the highest concentration of AcTCH (i.e. 0.50 mM).

G) Inhibitor Solutions

All inhibitor solutions were made in 50% aqueous acetonitrile with a stock concentration of $5x10^{-3}$ M.

H) Kinetic Studies

The esterase activity of human serum BuChE and human erythrocyte AChE was studied using a modified Ellman assay (32). In a quartz cuvette of 1-cm path length the following reaction components were combined and mixed, to give a final volume of 3.0 mL: 2.7 mL of buffered DTNB (pH 8.0), 0.1 mL of enzyme (AChE or BuChE) and, 0.1 mL of either 50% aqueous acetonitrile or inhibitor in 50% aqueous acetonitrile. The reaction was initiated by the addition of substrate (AcTCH or BuTCH), and was analyzed at room temperature using a Milton-Ray™ UV-visible spectrophotometer set at 412 nm. The change of absorbance was recorded at 5-second intervals for a period of one minute. The Abs/min values represent the rate of hydrolysis of the substrate by the enzyme.

I) Determination of Inhibitor Specificity

AChE and BuChE were exposed to a number of serial dilutions of each compound ($1.7x10^{-4}$-$1.7x10^{-9}$ M), at the highest substrate concentration (0.50 mM). Inhibition profiles were generated by plotting the rate of substrate hydrolysis (Abs/min) versus the log of the inhibitor concentration.

J) Generation of Lineweaver-Burk plots

Lineweaver-Burk plots were produced by plotting the inverse of the rate (Abs/min) versus the inverse of the substrate concentration. Three separate runs were performed, each employing a different inhibitor concentration (one without inhibitor and two carried out in the presence of different inhibitor concentrations). The inhibitor concentrations used were selected from the inhibition profile described above. Each run consisted of a series of assays in which the concentration of enzyme and inhibitor were held constant while the substrate concentration was varied (i.e.0.50 mM - 0.0313 mM). K_m and V_{max} values, in addition to the type of inhibition were obtained from the Lineweaver-Burk plots.

K) Determination of the Inhibition constant (K_i)

The strength of the inhibition, the inhibition constant (K_i), was determined by plotting the slope of each of the Lineweaver-Burk lines against their respective inhibitor concentrations. Each K_i value was obtained from the x-intercept of its respective graph. The K_i values provided represent the average of two values.

Results and Discussion:

It has been shown that the active site in cholinesterases is at the bottom of a "gorge" which is lined by aromatic amino acid residues, 12 in AChE and 6 in BuChE. Some inhibitors bind to a peripheral site close to the gorge to exert their action. In the case of the phenothiazine derivatives of the present invention, the

nature of inhibition is generally mixed non-competitive suggesting that these compounds most likely bind to the peripheral site near the active-site gorge. It is possible that the phenothiazine moiety binds at this site and the nitrogen containing side chain binds to the amino acid residues in the gorge in a reversible manner. Compounds 15 and 27 display competitive inhibition towards BuChE and AChE.

The difference in K_i values (Table 2) for the different compounds may be due to binding properties of the side chains.

TABLE 2

AChE and BuChE Inhibition Results

Compd.	K_I BuChE (M)	K_I AChE (M)
Phenothiazine	1.2×10^{-5}	Insignificant inhibition
Ethopropazine	2.4×10^{-7}	Insignificant inhibition
1	3.9×10^{-5}	1.1×10^{-4}
2	9.3×10^{-6}	7.9×10^{-5}
3	7.4×10^{-7}	Slight inhibition
4	9.3×10^{-7}	Slight inhibition
5	3.6×10^{-7}	5.7×10^{-5}
6	5.5×10^{-7}	2.6×10^{-5}
7	2.0×10^{-7}	3.5×10^{-5}
8	1.7×10^{-8}	6.9×10^{-7}

Table 2 - continued

Compd.	K_I BuChE (M)	K_I AChE (M)
9	5.7×10^{-7}	1.0×10^{-4}
10	8.0×10^{-6}	Insignificant inhibition
11	7.9×10^{-6}	Insignificant inhibition
12	1.2×10^{-6}	Insignificant inhibition
13	6.9×10^{-7}	4.2×10^{-5}
14	5.5×10^{-7}	2.6×10^{-5}
15	1.92×10^{-6}	1.74×10^{-4}
16	9.6×10^{-7}	2.0×10^{-5}
17	5.9×10^{-7}	Insignificant inhibition
18	2.4×10^{-5}	Insignificant inhibition
19	6.97×10^{-6}	4.51×10^{-6}
20	1.1×10^{-5} (K_i represents a single value)	
21	No data	Insignificant inhibition
22	1.3×10^{-5}	1.12×10^{-4}
23	No data	Insignificant inhibition
24	2.8×10^{-6}	Insignificant inhibition
25	4.7×10^{-6}	Insignificant inhibition

Table 2 - continued

Compd.	K_I BuChE (M)	K_I AChE (M)
26	4.0×10^{-6}	Insignificant inhibition
27	2.58×10^{-5}	8.38×10^{-5}
28	1.12×10^{-5}	Significant inhibition but no data
29	1.04×10^{-5}	Insignificant inhibition
30	2.56×10^{-5}	Insignificant inhibition
31	2.78×10^{-5}	Insignificant inhibition
32	1.2×10^{-5}	Insignificant inhibition
33	1.18×10^{-5}	Insignificant inhibition
34	1.06×10^{-5}	Insignificant inhibition
35	3.27×10^{-5}	Insignificant inhibition
36	2.04×10^{-6}	Insignificant inhibition
37	2.98×10^{-6}	Insignificant inhibition
38	5.87×10^{-6}	Insignificant inhibition
39	9.82×10^{-7}	Insignificant inhibition

Table 2 - continued

Compd.	K_I BuChE (M)	K_I AChE (M)
40	5.6×10^{-7}	Insignificant inhibition
41	1.42×10^{-6}	Insignificant inhibition
42	6.44×10^{-6}	Insignificant inhibition
43	9.56×10^{-7}	2.0×10^{-5}

While the invention has been described in particular, one skilled in the art understands that variations from the particularly described embodiments may be done without departing from the spirit and scope of the invention described and claimed herein.

References:

1. Mayeux R., and Sano M. (1999) Treatment of Alzheimer's disease. *New England Journal of Medicine* 341: 1670-1679.

2. Patterson, C. J. S, Gauthier, S., Berman, H., Cohen, C.A., Feighter, J.W., Feldman, H. and Hogan, D.B. 1999. The recognition, assessment and management of dementing disorders: conclusions from the Canadian Consensus Conference on Dementia. *Canadian Medical Association Journal* 160: S1-S15.

3. Cummings, J.L., Vinters, H.V., Cole, G.M. and Khachaturian, Z.S. 1998. Alzheimer's disease: Etiologies, pathophysiology, cognitive reserve, and treatment opportunities. *Neurology* 51: S2-S17.

4. Mirra, S.S., Heyman, A., McKeel, D., Sumi, S.M., Crain, B.J., Brownlee, L.M., Vogel, F.S., Hughes, J.P., Van Belle, G., Berg, L., and participating CERAD neuropathologists. 1991. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 41: 479-486.

5. Bartus, R.T., Dean, R.L., Beer, B., and Lippa, A.S. 1982. The cholinergic hypothesis of geriatric memory dysfunction. *Science* 217: 408-417.

6. Coyle, J.T., Price, D.L. and DeLong, M.R. 1983. Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science* 219: 1184-1190.

7. Geula, C., and Mesulam, M-M. (1995) Cholinesterases and the pathology of Alzheimer disease. *Alzheimer Disease and Associated Disorders* 2: 23-28.

8. Mesulam, M-M. and Geula, C.(1994) Butyrylcholinesterase reactivity differentiates the amyloid plaques of aging from those of dementia. *Annals of Neurology*. 36: 722-727.

9. Geula, C., and Mesulam, M-M. (1989) Special properties of cholinesterases in the cerebral cortex of Alzheimer's disease. *Brain Research*. 498: 185-189.

10. Greig, N.H., Lahiri, D.K., Soncrant, T.T., Utsuki, T., Yu, O.S., Shaw, K.T.Y., Holloway, H.W., Myer, R.C., Wallace, W.C., Haroutunian, V. and Ingram, D.K. 1998. Novel, selective butyrylcholinesterase (BChE) inhibitors for the treatment of Alzheimer's disease (AD). *Society for Neuroscience Abstracts* 24: 728.

11. Hardy, J. 1997 Amyloid, the presenilins and Alzheimer's disease. *Trends in Neuroscience* 20: 154-159.

12. Selkoe, D.J. 1991. The molecular pathology of Alzheimer's disease. *Neuron* 6: 487-498.

13. Guillozet, A.L., Smiley, J.F., Mash, D.C. and Mesulam, M-M. 1997. Butyrylcholinesterase in the life cycle of amyloid plaques. *Annals of Neurology* 42: 909-918.

14. Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. and Ito, H. 1988. Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. *Nature* 331: 530-532.

15. Tanzi, R.E., McClatchey, A.I., Lamperti, E.D., Villa-Komaroff, L., Gusella, J.F. and Neve, R.L. 1988. Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature* 331:528-530.

16. Haas, C. and Selkoe, D.J. 1993. Cellular processing of ß-amyloid precursor protein and the genesis of amyloid ß-peptide. *Cell* 75: 1039-1042.

17. Hardy, J. 1997 Amyloid, the presenilins and Alzheimer's disease. *Trends in Neuroscience* 20: 154-159.

18. Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Culwell, A.R., Oltersdorf, T., McClure, D. and Ward, P.J. 1990. Cleavage of amyloid peptide during constitutive processing of its precursor. *Science* 248: 1122-1124.

19. Tomita, S., Kirino, Y. and Suzuki, T. 1998. A basic amino acid in the cytoplasmic domain of Alzheimer's beta-amyloid precursor protein (APP) is essential for cleavage of APP at the alpha-site. *Journal of Biological Chemistry* 273: 19304-19310.

20. Anderson, J.P., Esch, F.S., Keim, P.S., Sambamurti, K., Lieberburg, I. and Robakis, N.K. 1991. Exact cleavage site of Alzheimer amyloid precursor in neuronal PC-12 cells. *Neuroscience Letters* 128: 126-128.

21. Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Whaley, J., Swindlhurst, C., McCormak, R., Wolfert, R., Selkoe, D.J., Lieberburg, I. and Schenk, D. 1992. Isolation and quantitation of soluble Alzheimer's ß-peptide from biological fluids. *Nature* 359: 325-327.

22. Hooper, N.M., Karran, E.H. and Turner, A.J. 1997. Membrane protein secretases. *Biochemical Journal* 321: 265-279.

23. Worthington, V. 1993. Enzymes and related biochemicals. *Worthington Enzyme Manual*. Worthington Biochemical Corporation. Freehold, New Jersey.

24. Minn, A., Schubert, M., Neiss, W.F. and Muller-Hill, B. 1998. Enhanced GFAP expression in astrocytes of

transgenic mice expressing the human brain-specific trypsinogen IV. *Glia*. 22: 338-347.

25. De Serres, M., Sherman, D., Chestnut, W., Merrill, B.M., Viveros, O.H. and Diliberto, E.J. Jr. (1993) Proteolysis at the secretase and amyloidogenic cleavage sites of the beta-amyloid precursor protein by acetyl cholinesterase and butyrylcholinesterase using model peptide substrates. *Cell Molecular and Neurobiology* 13: 279-287.

26. Meckelein, B., Marshall, D.C.L., Conn, K-J., Pietopaolo, M., Van Nostrand, W. and Abraham, C. (1998) Identification of a novel serine protease-like molecule in human brain. *Brain Research Molecular Brain Research* 55: 181-197.

27. Wiegand, U., Corbach, S., Minn, A., Kang, J. and Muller-Hill, B. 1993. Cloning of the cDNA encoding human brain trypsinogen and characterization of its product. *Gene* 136: 167-175.

28. Boopathy, R. and Balasubramanian, A.S. 1987. A peptidase activity exhibited by human serum pseudocholinesterase. *European. Journal of Biochemistry* 162: 191-197.

29. Lockridge, O. 1982. Substance P hydrolysis by human serum cholinesterase. *Journal of Neurochemistry* 39: 106-110.

30. Darvesh, S., Kumar, R., Roberts, S., Walsh, R. and Martin, E. (2001) Butyrylcholinesterase-Mediated

Enhancement of the Enzymatic Activity of Trypsin. *Cellular and Molecular Neurobiology*, accepted.

31. Darvesh, S., Kumar, R. and Martin, E. (1999) Enzyme kinetics of butyrylcholinesterase and trypsin: Implications in Alzheimer's disease. *Canadian Journal Neurological Sciences*, 26, 546-547.

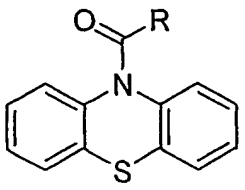
32. Ellman, G.L., Courtney, K.D., Andres, V. Jr. and Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetyl cholinesterase activity. *Biochemical Pharmacology*, 7, 88-95.

33. Small, D.H., Michaelson, S. and Sberna, G. (1996) Non-classical actions of cholinesterases: Role in cellular differentiation, tumorigenesis and Alzheimer's disease. *Neurochemistry International*, 28, 453-483.

34. Layer, P.G. (1995) Non-classical roles of cholinesterases in the embryonic brain and possible links to Alzheimer disease *Alzheimer Disease and Associated Disorders*, 9, 29-36.

CLAIMS:

1. A compound of the formula (I):



(I)

wherein R is:

(a) a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by phenyl, halo or $-NR_1R_2$, wherein R_1 and R_2 are independently H, a branched or straight chain (C_1-C_6)alkyl group or R_1 and R_2 together with the nitrogen atom to which they are bonded form a 5- or 6-membered ring;

(b) phenyl; or

(c) $-NR_3R_4$, wherein R_3 and R_4 are independently;

(i) H,

(ii) a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by (C_1-C_4)alkoxy, phenyl or $-NR_5R_6$, wherein R_5 and R_6 are independently H, a branched or straight chain (C_1-C_4)alkyl group, phenothiazine-10-carbonyl or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a 5- or 6-membered ring,

(iv) a (C_5-C_6)cycloalkyl group, or

(iv) R_3 and R_4 together with the nitrogen atom to which they are bonded form pyrrolidino, piperidino, morpholino, piperazino or 4-methylpiperazino,

or a pharmacologically acceptable salt thereof.

2. The compound according to claim 1, or a pharmacologically acceptable salt thereof, wherein R is a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by phenyl, or R is $-NR_3R_4$.

3. The compound according to claim 1, or a pharmacologically acceptable salt thereof, wherein R is $-NR_3R_4$, a straight chain (C_1-C_4)alkyl group or a straight chain (C_1-C_4)alkyl group substituted by phenyl.

4. The compound according to any one of claims 1 to 3, or a pharmacologically acceptable salt thereof, wherein R_3 and R_4 are independently H or a branched or straight chain (C_1-C_6)alkyl group unsubstituted or substituted by $-NR_5R_6$.

5. The compound according to any one of claims 1 to 3, or a pharmacologically acceptable salt thereof, wherein one of R_3 or R_4 is H and the other is a branched or straight chain (C_1-C_4)alkyl group substituted by $-NR_5R_6$.

6. The compound according to any one of claims 1 to 5, or a pharmacologically acceptable salt thereof, wherein R_5 and R_6 are independently H, a branched or straight chain (C_1-C_4)alkyl group or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a saturated 5- or 6-membered ring.

7. The compound according to any one of claims 1 to 5, or a pharmacologically acceptable salt thereof, wherein R_5 and R_6 are independently a branched or straight chain (C_1-C_4)alkyl group or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a saturated 5- or 6-membered ring.

8. The compound according to claim 1, or a pharmacologically acceptable salt thereof, wherein R is methyl, ethyl, n-propyl, $-CH_2$-phenyl, $-(CH_2)_2$-phenyl, $-NH-(CH_2)_2-NR_5R_6$ or $-NH-CH_2-C(CH_3)_2-CH_2-R_5R_6$, wherein R_5 and R_6 are methyl, ethyl or R_5 and R_6 taken together with the nitrogen atom to which they are bonded form a pyrrolidino or a piperidinyl ring.

9. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is 10-acetyl-10*H*-phenothiazine.

10. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is 10-butyryl-10*H*-phenothiazine.

11. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is 10-(phenylacetyl)-10*H*-phenothiazine.

12. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is 10-(3-phenylpropanoyl)-10*H*-phenothiazine.

13. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is

N-[2-(dimethylamino)ethyl]-10H-phenothiazine-10-carboxamide.

14. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is N-[2-(diethylamino)ethyl]-10H-phenothiazine-10-carboxamide.

15. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is N-(2-pyrrolidin-1-ylethyl)-10H-phenothiazine-10-carboxamide.

16. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is N-(2-piperidin-1-ylethyl)-10H-phenothiazine-10-carboxamide.

17. The compound according to claim 1, or a pharmacologically acceptable salt thereof, which is N-[3-(dimethylamino)-2,2-dimethylpropyl]-10H-phenothiazine-10-carboxamide.

18. A pharmaceutical composition comprising a therapeutically effective amount of a compound as defined in any one of claims 1 to 17, or a pharmacologically acceptable salt thereof, together with a pharmaceutically acceptable carrier, diluent or excipient.

19. The composition according to claim 18 for use in modulating activity of a serine hydrolase enzyme.

20. The composition according to claim 19, wherein the serine hydrolase enzyme is a cholinesterase and the activity of the cholinesterase is inhibited.

21. The composition according to claim 20, wherein the cholinesterase is butyrylcholinesterase (BuChE).

22. The composition according to claim 20, wherein the cholinesterase acetylcholinesterase (AChE).

23. The composition according to any one of claims 18 to 22 for use in treating Alzheimer's disease.

24. The composition according to any one of claims 18 to 23 for use in a mammal.

25. The composition according to claim 24, wherein the mammal is a human.

26. Use of a therapeutically effective amount of a compound as defined in any one of claims 1 to 17, or a pharmacologically acceptable salt thereof, for modulating activity of a serine hydrolase enzyme.

27. Use of a therapeutically effective amount of a compound as defined in any one of claims 1 to 17, or a pharmacologically acceptable salt thereof, for preparing a medicament for modulating activity of a serine hydrolase enzyme.

28. The use according to claim 26 or 27, wherein the serine hydrolase enzyme is a cholinesterase and the activity of the cholinesterase is inhibited.

29. The use according to claim 28, wherein the cholinesterase is butyrylcholinesterase (BuChE).

30. The use according to claim 28, wherein the cholinesterase acetylcholinesterase (AChE).

31. The use according to any one of claims 26 to 30 for treating Alzheimer's disease.

32. The use according to any one of claims 26 to 31 in a mammal.

33. The use according to claim 32, wherein the mammal is a human.

34. A commercial package comprising a therapeutically effective amount of a compound as defined in any one of claims 1 to 17, or a pharmacologically acceptable salt thereof, together with instructions for its use in modulating activity of a serine hydrolase enzyme.

35. The commercial package according to claim 34, wherein the serine hydrolase enzyme is a cholinesterase and the activity of the cholinesterase is inhibited.

36. The commercial package according to claim 35, wherein the cholinesterase is butyrylcholinesterase (BuChE).

37. The commercial package according to claim 35, wherein the cholinesterase acetylcholinesterase (AChE).

38. The commercial package according to any one of claims 34 to 37, wherein the instructions are for use in treating Alzheimer's disease.

39. The commercial package according to any one of claims 34 to 37, wherein the instructions are for use in a mammal.

40. The commercial package according to claim 39, wherein the mammal is a human.

41. A method of modulating activity of a serine hydrolase enzyme in a mammal, comprising administering to the mammal a composition as defined in claim 18.

42. The method according to claim 41, wherein the serine hydrolase enzyme is a cholinesterase and the activity of the cholinesterase is inhibited.

43. The method according to claim 42, wherein the cholinesterase is butyrylcholinesterase (BuChE).

44. The method according to claim 42, wherein the cholinesterase acetylcholinesterase (AChE).

45. The method according to any one of claims 41 to 44 for treating Alzheimer's disease.

46. The method according to any one of claims 41 to 45, wherein the mammal is a human.

(v) a (C_5-C_6)cycloalkyl group, or

(iv) R_3 and R_4 together with the nitrogen atom to which they are bonded form pyrrolidino, piperidino, morpholino, piperazino or 4-methylpiperazino,

or a pharmacologically acceptable salt thereof,

for use in the treatment of Alzheimer's disease and other conditions. Compounds of the formula (I) modulate the activity of serine hydrolase enzymes, for example, they are cholinesterase inhibitors.

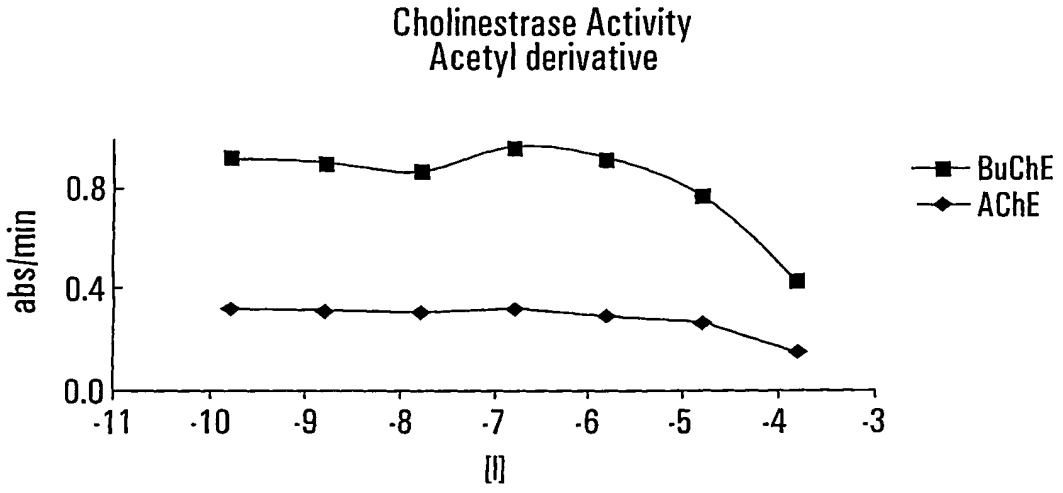

Cholinestrase Activity
Acetyl derivative
abs/min
0.0
0.4
0.8
-11
-10
-9
-8
-7
-6
-5
-4
-3
[I]
BuChE
AChE

FIG. 1A

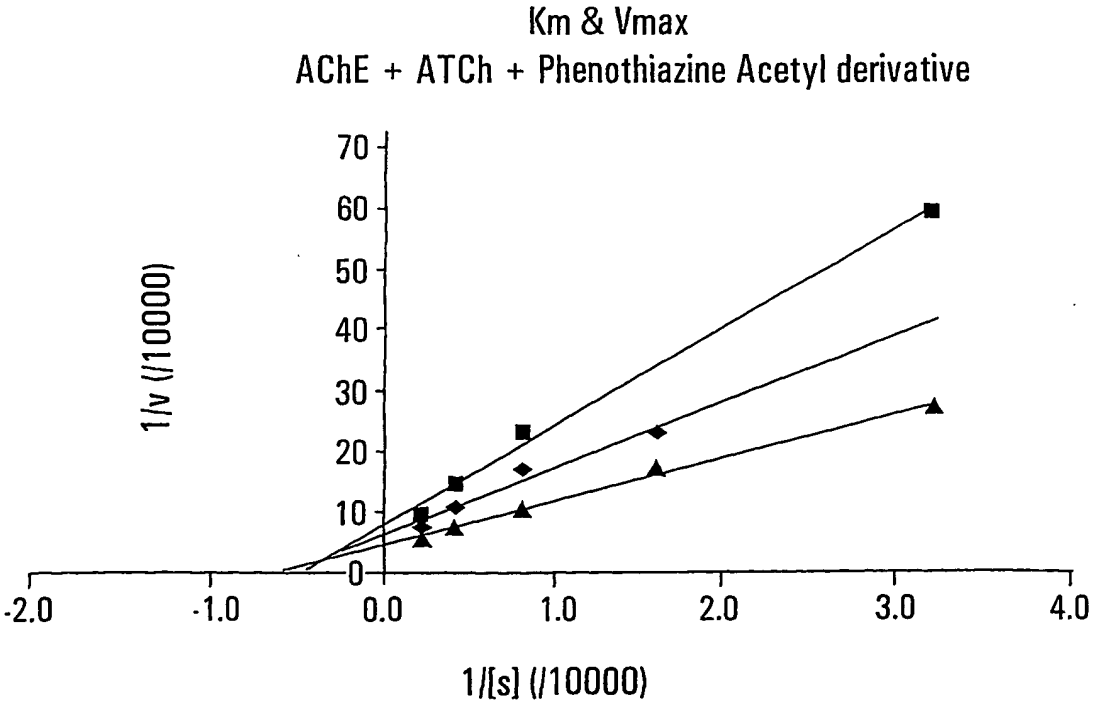

Km & Vmax
AChE + ATCh + Phenothiazine Acetyl derivative
1/v (/10000)
0
10
20
30
40
50
60
70
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 1B

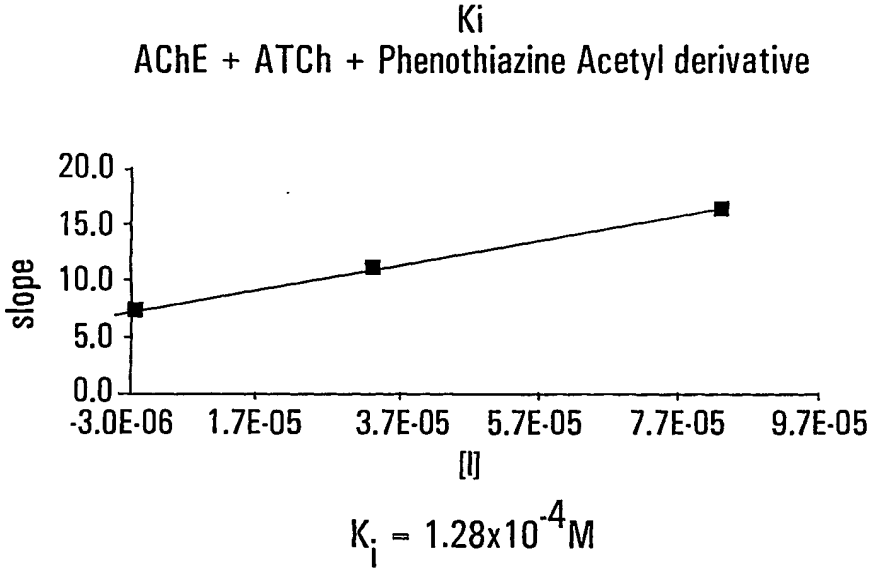

Ki
AChE + ATCh + Phenothiazine Acetyl derivative
20.0
15.0
10.0
5.0
0.0
slope
-3.0E-06
1.7E-05
3.7E-05
5.7E-05
7.7E-05
9.7E-05
[I]
$K_i = 1.28x10^{-4}M$

FIG. 1C

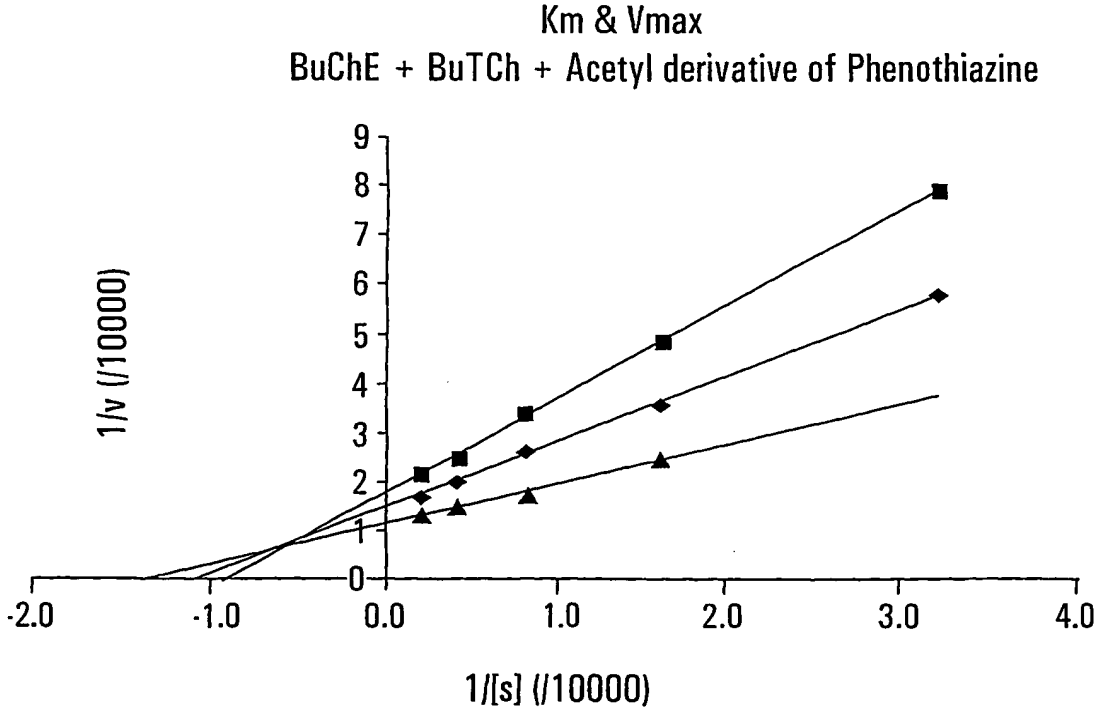

Km & Vmax
BuChE + BuTCh + Acetyl derivative of Phenothiazine
9
8
7
6
5
4
3
2
1
0
1/v (/10000)
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 1D

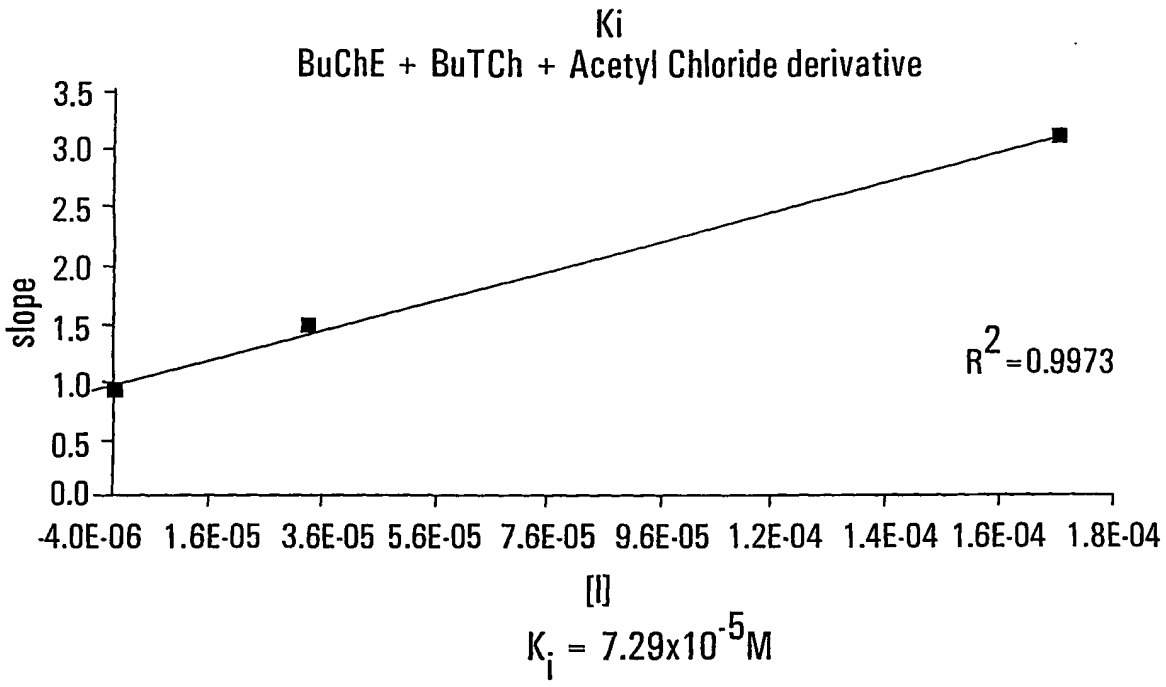

Ki
BuChE + BuTCh + Acetyl Chloride derivative
slope
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
R^2=0.9973
-4.0E-06 1.6E-05 3.6E-05 5.6E-05 7.6E-05 9.6E-05 1.2E-04 1.4E-04 1.6E-04 1.8E-04
[I]
$K_i = 7.29x10^{-5}M$

FIG. 1E

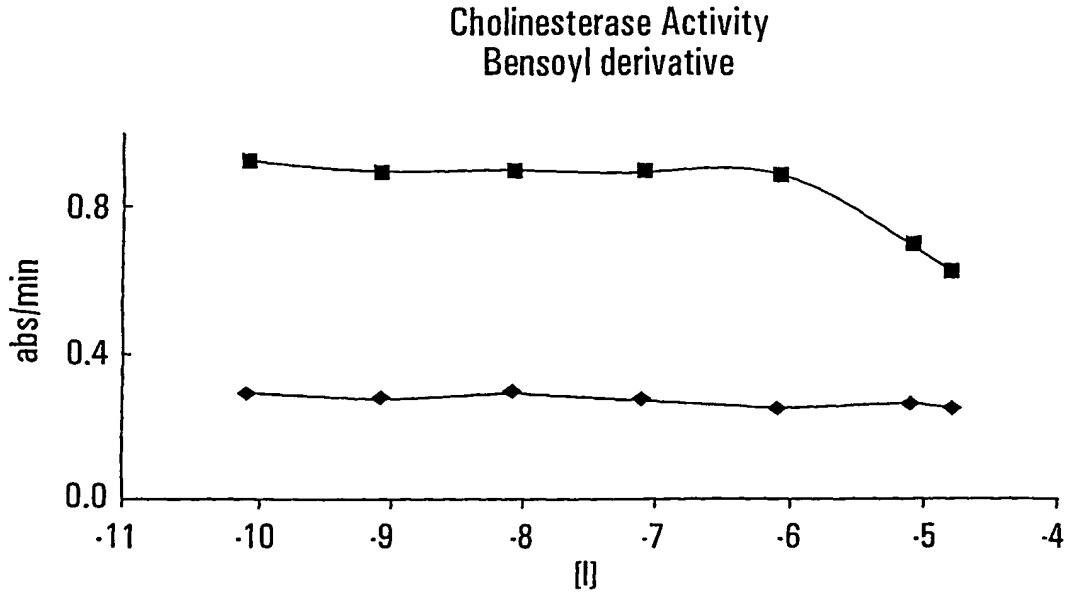

Cholinesterase Activity
Bensoyl derivative
abs/min
0.8
0.4
0.0
-11 -10 -9 -8 -7 -6 -5 -4
[I]

FIG. 2A

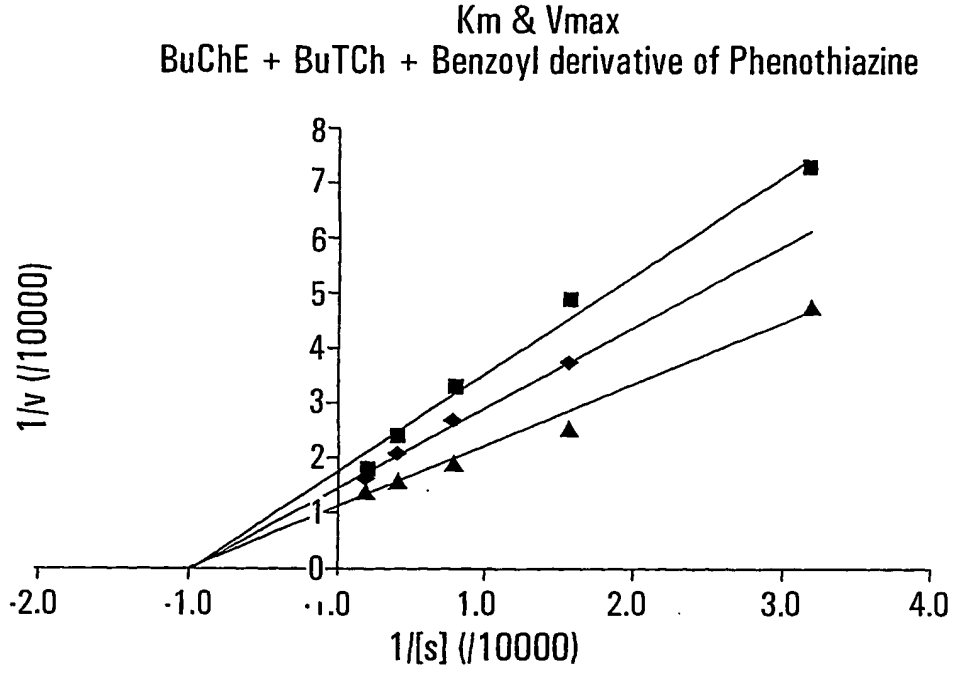

Km & Vmax
BuChE + BuTCh + Benzoyl derivative of Phenothiazine
1/v (/10000)
1/[s] (/10000)
8
7
6
5
4
3
2
1
0
-2.0
-1.0
1.0
2.0
3.0
4.0

FIG. 2B

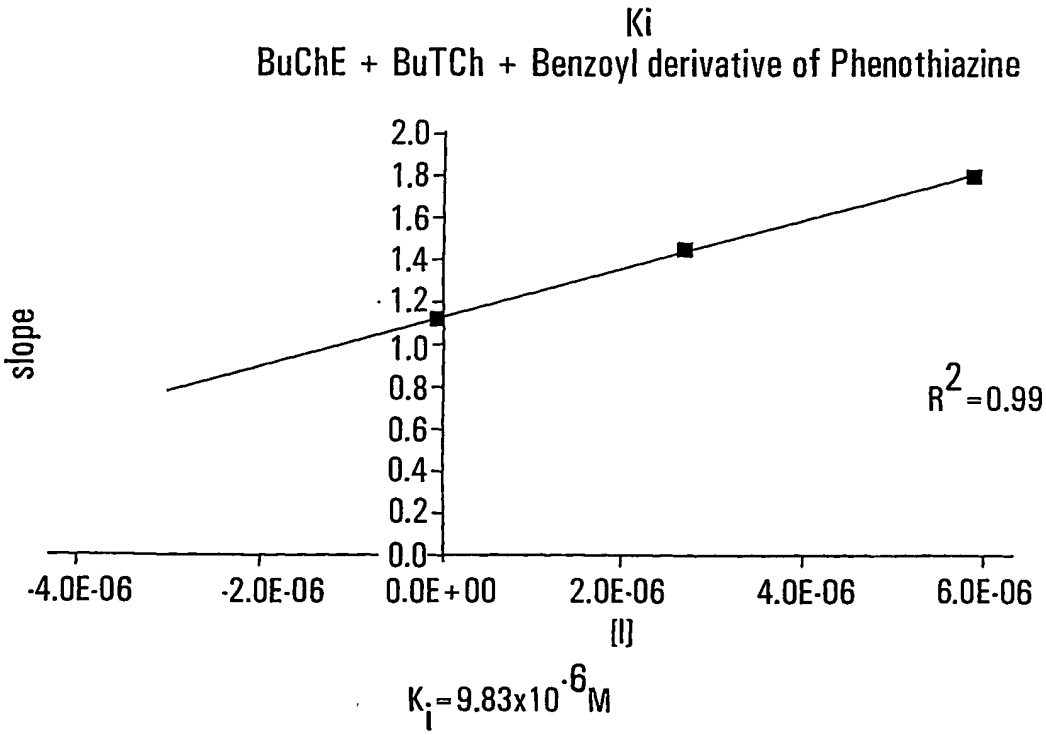

Ki
BuChE + BuTCh + Benzoyl derivative of Phenothiazine
slope
[I]
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-4.0E-06
-2.0E-06
0.0E+00
2.0E-06
4.0E-06
6.0E-06
R2=0.99
Ki=9.83x10-6M

FIG. 2C

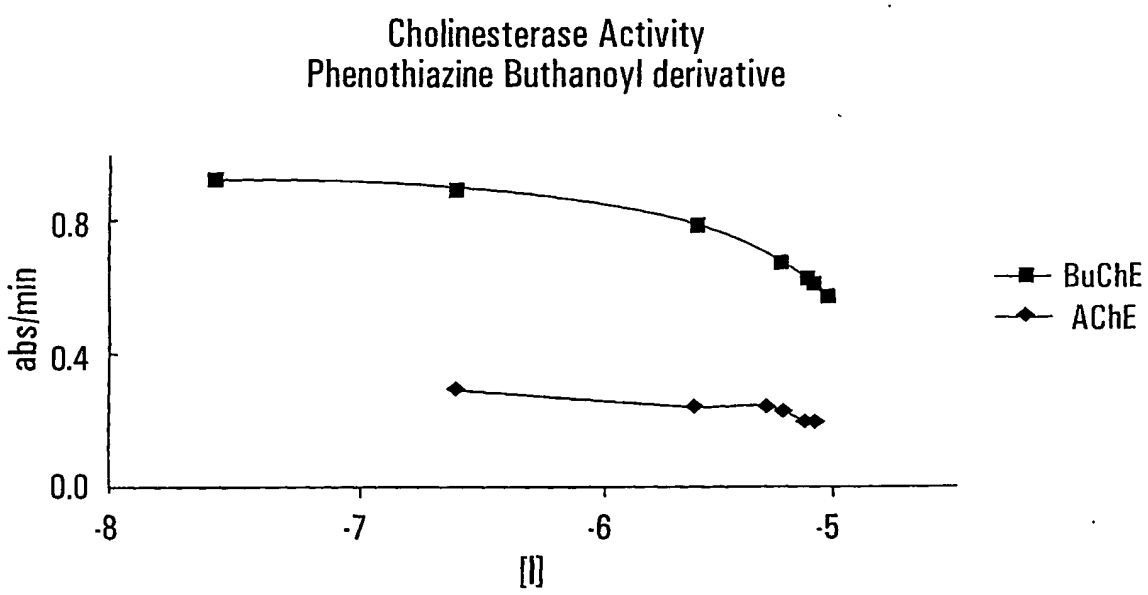

Cholinesterase Activity
Phenothiazine Buthanoyl derivative
abs/min
0.0
0.4
0.8
-8
-7
-6
-5
[I]
BuChE
AChE

FIG. 3A

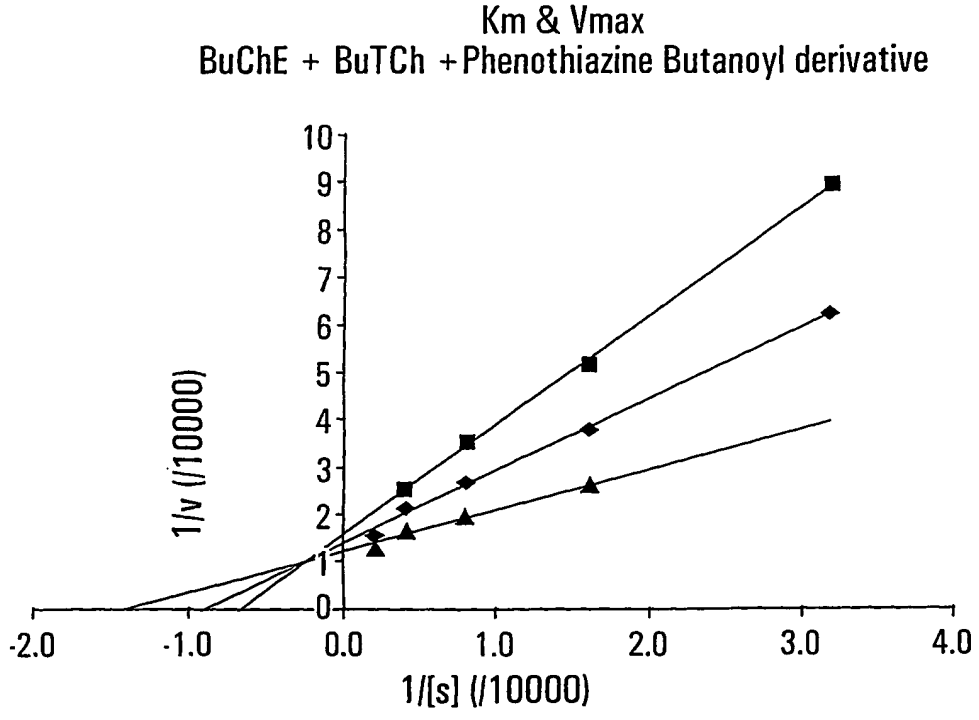
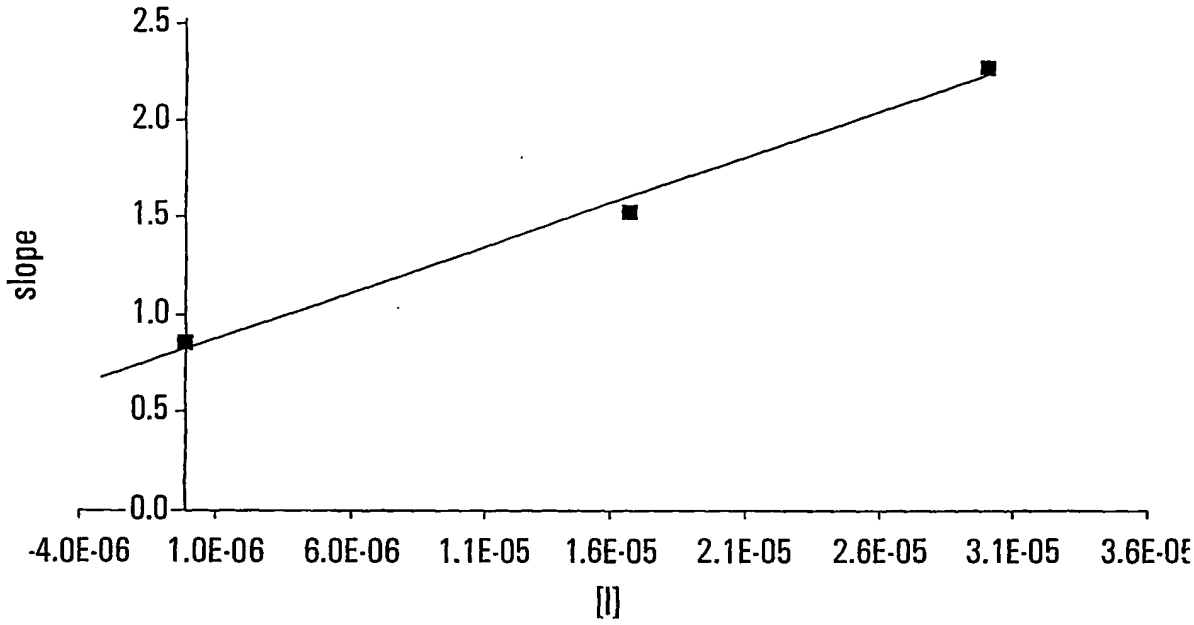

Km & Vmax
BuChE + BuTCh + Phenothiazine Butanoyl derivative
1/v (/10000)
0
1
2
3
4
5
6
7
8
9
10
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 3B

Ki

BuChE + BuTCh + Phenothiazine Butanoyl derivative



$K_i = 1.68 \times 10^{-5} M$

FIG. 3C

Km & Vmax

AChE + ATCh + Phenothiazine Butanoyl derivative

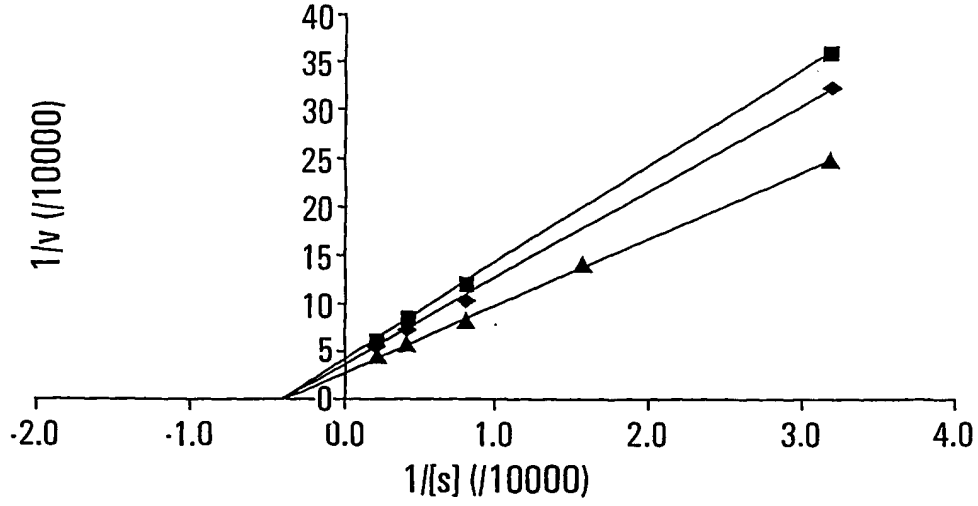


FIG. 3D

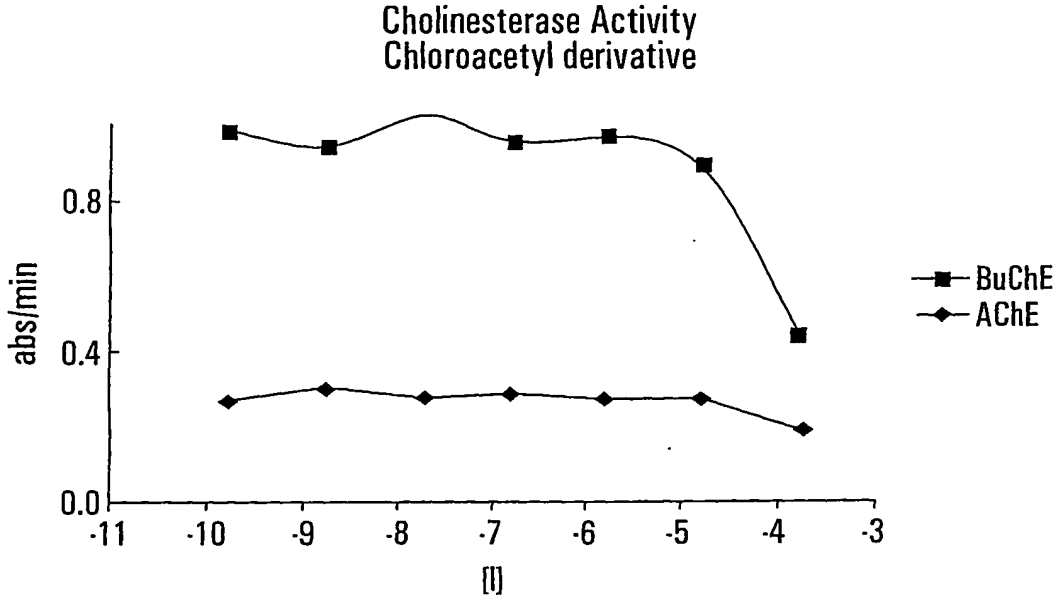

Cholinesterase Activity
Chloroacetyl derivative
abs/min
0.0
0.4
0.8
-11
-10
-9
-8
-7
-6
-5
-4
-3
[I]
BuChE
AChE

FIG. 4A

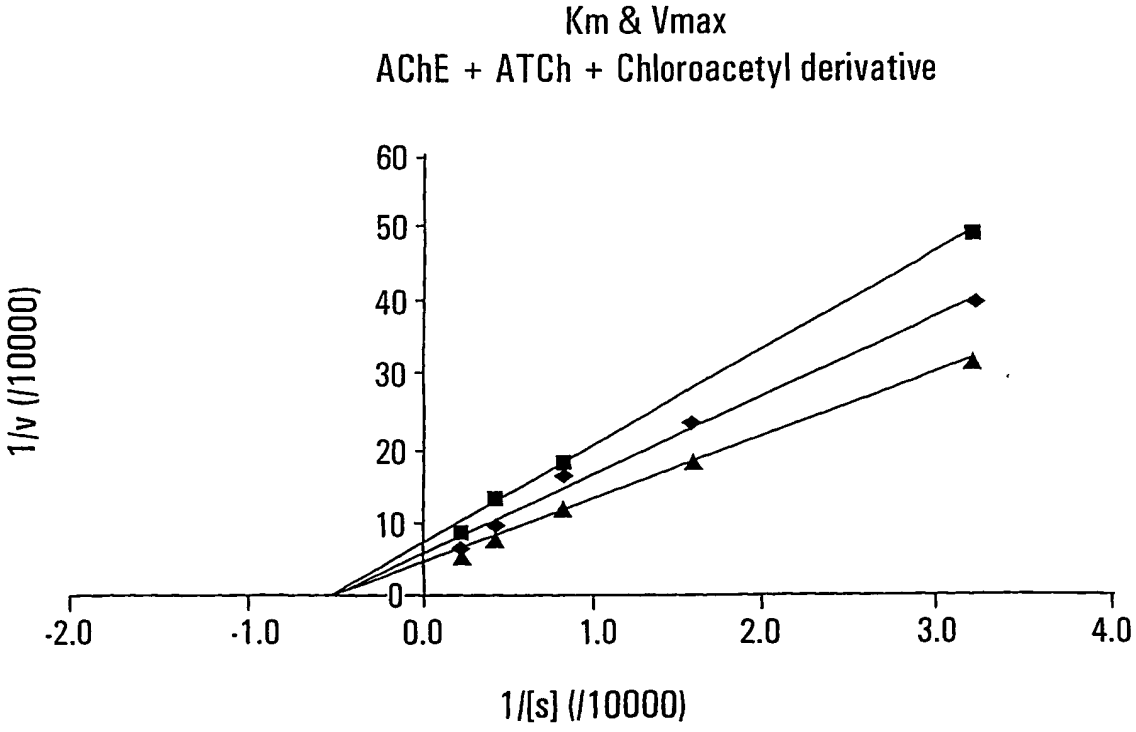

Km & Vmax
AChE + ATCh + Chloroacetyl derivative
1/v (/10000)
0
10
20
30
40
50
60
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 4B

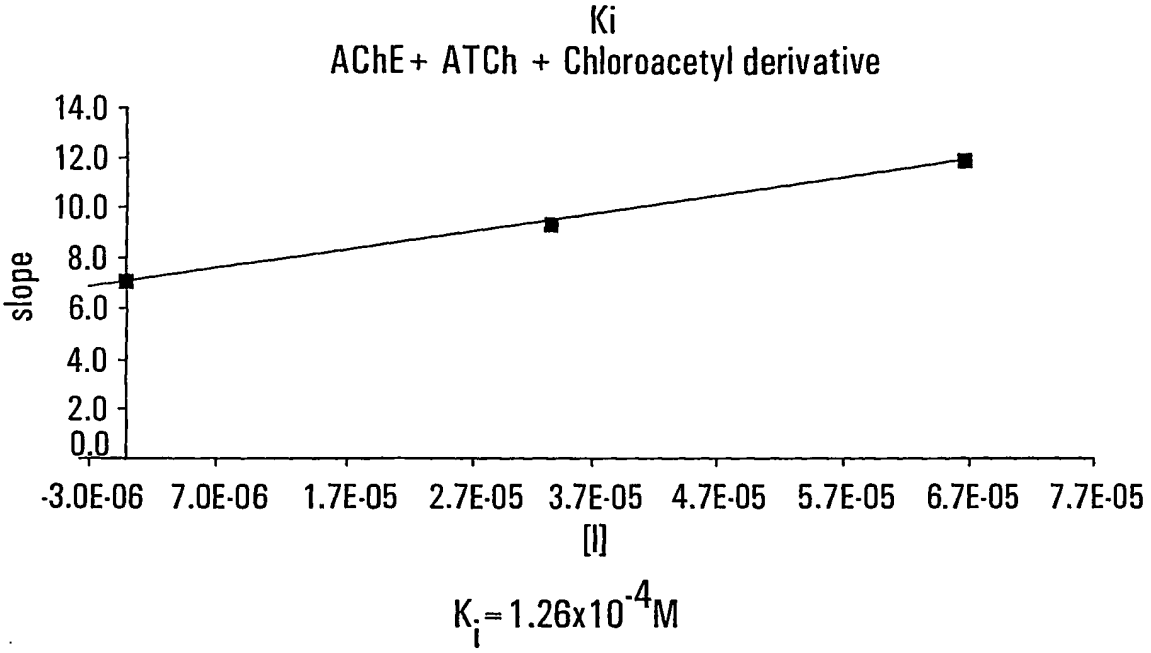

Ki
AChE+ ATCh + Chloroacetyl derivative
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
slope
-3.0E-06
7.0E-06
1.7E-05
2.7E-05
3.7E-05
4.7E-05
5.7E-05
6.7E-05
7.7E-05
[I]
$K_i = 1.26x10^{-4}M$

FIG. 4C

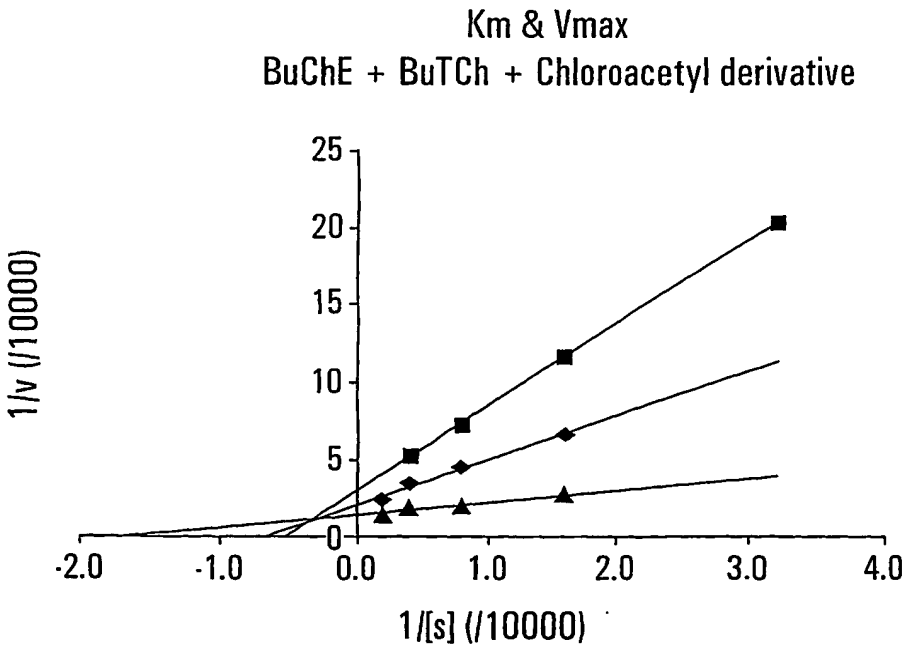
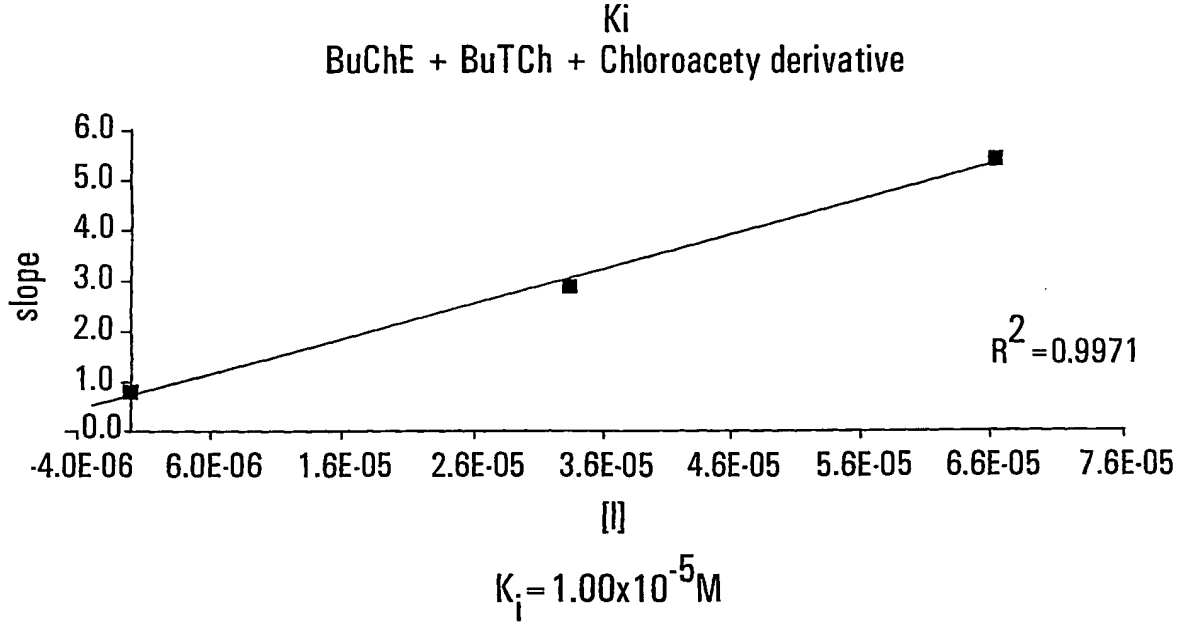

Km & Vmax
BuChE + BuTCh + Chloroacetyl derivative
25
20
15
10
5
0
1/v (/10000)
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 4D



$K_i = 1.00 \times 10^{-5} M$

FIG. 4E

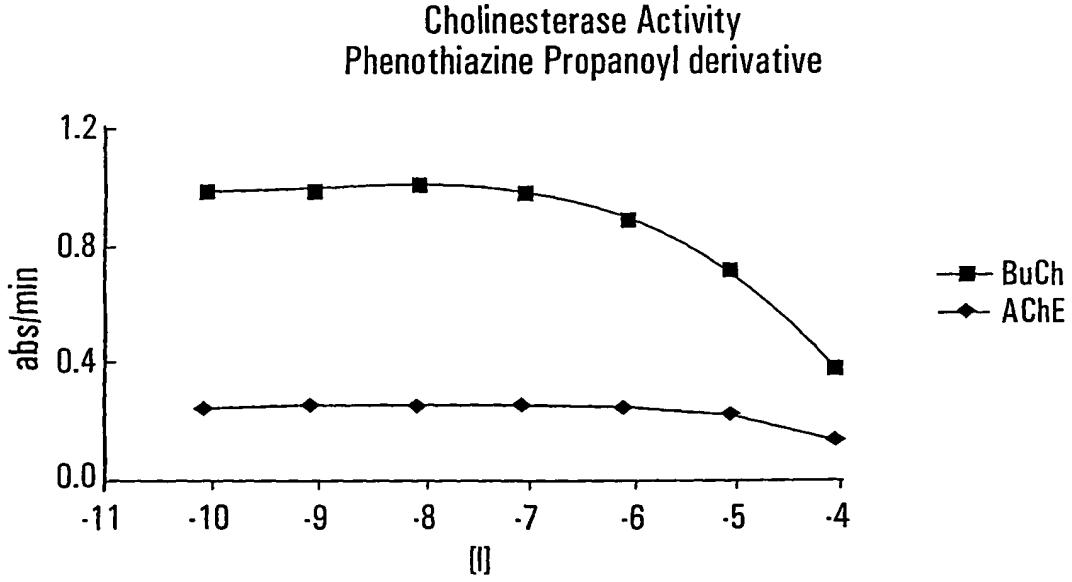


FIG. 5A

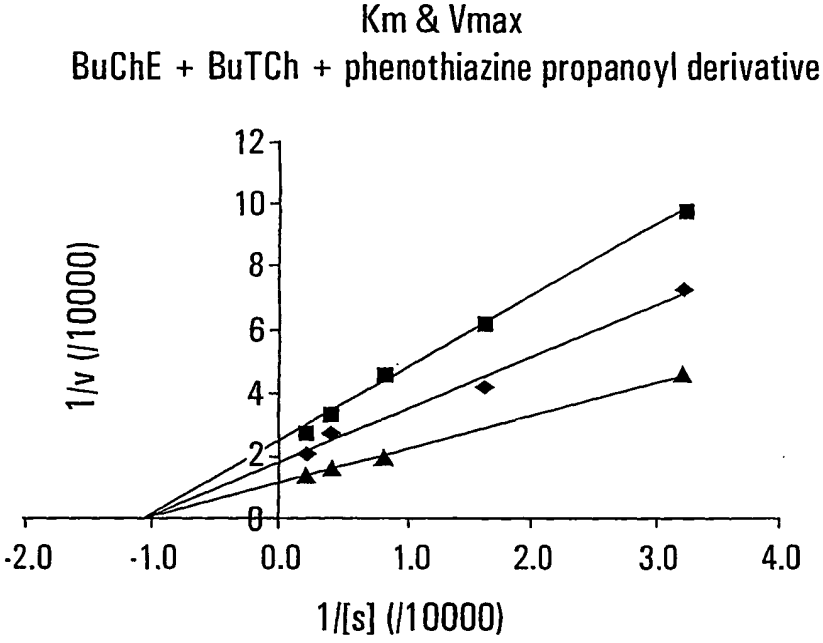

Km & Vmax
BuChE + BuTCh + phenothiazine propanoyl derivative
12
10
8
6
4
2
0
1/v (/10000)
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 5B

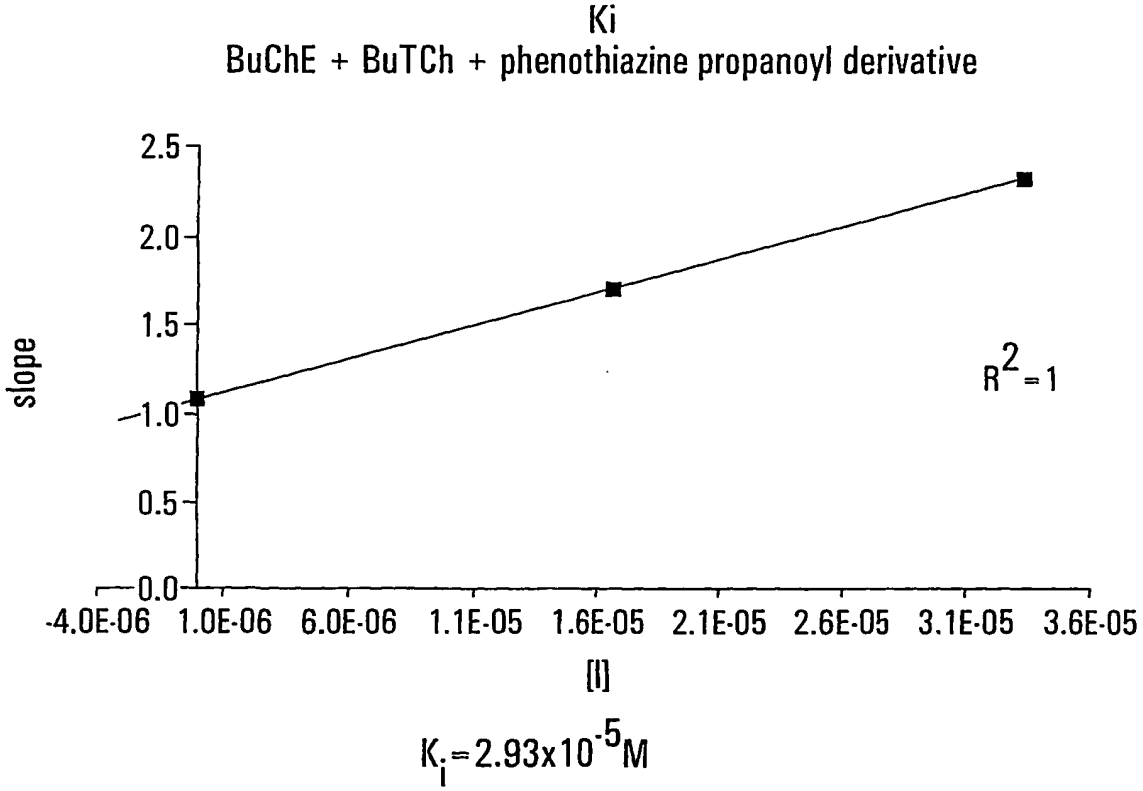

Ki
BuChE + BuTCh + phenothiazine propanoyl derivative
2.5
2.0
1.5
1.0
0.5
0.0
slope
$R^2 = 1$
-4.0E-06
1.0E-06
6.0E-06
1.1E-05
1.6E-05
2.1E-05
2.6E-05
3.1E-05
3.6E-05
[I]
$K_i = 2.93 \times 10^{-5} M$

FIG. 5C

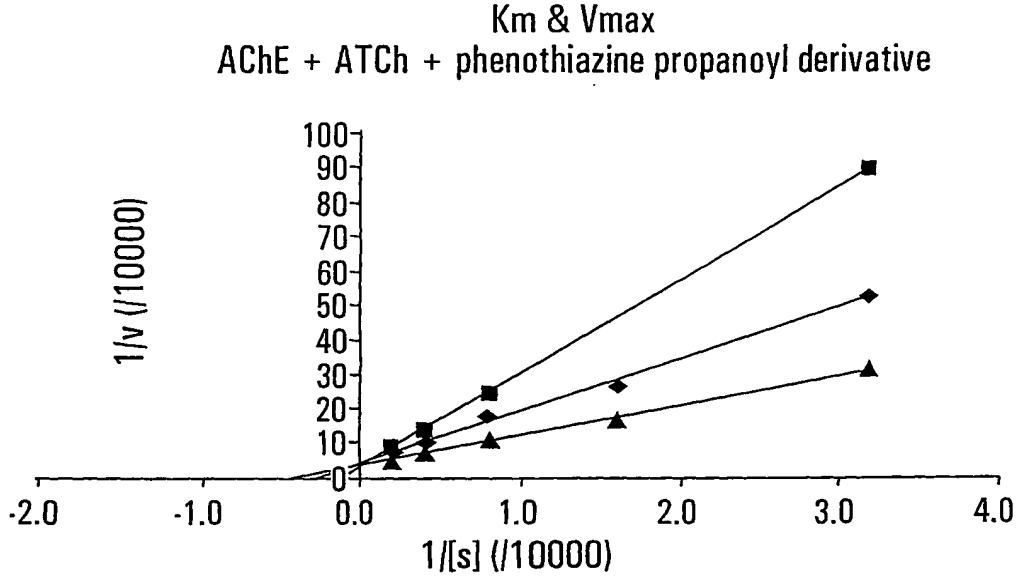
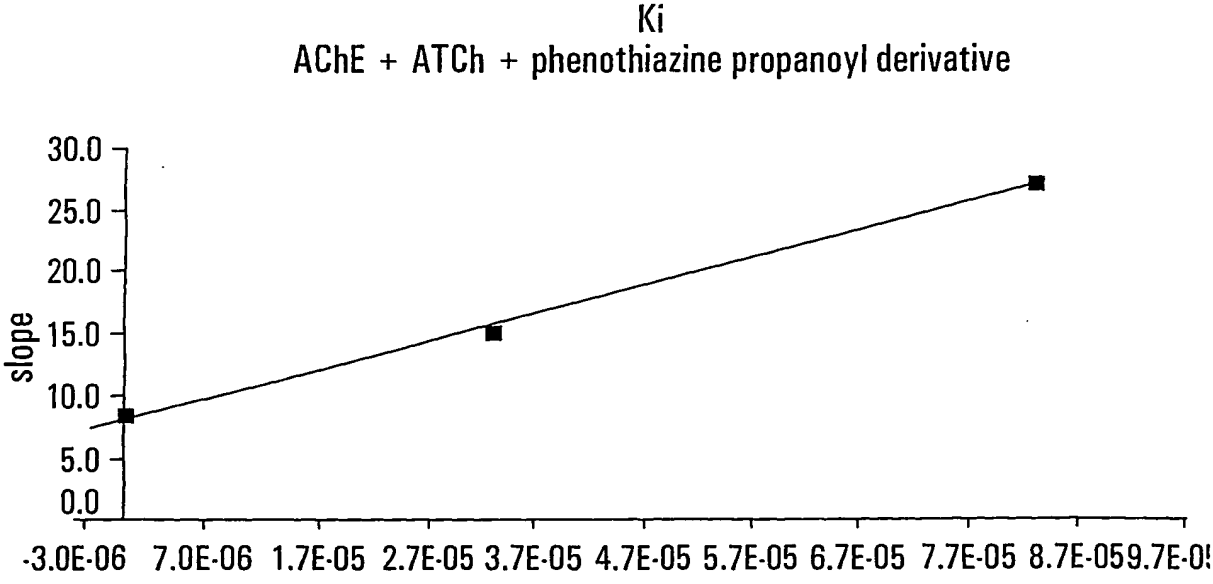


FIG. 5D



[1]

$K_i = 3.65 \times 10^{-5}$ M

FIG. 5E

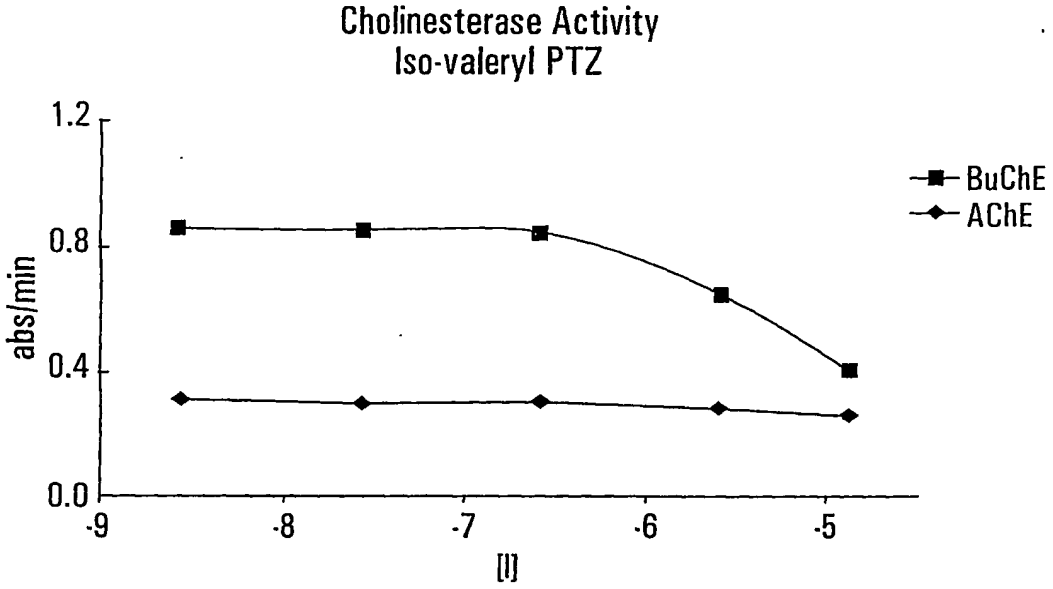

Cholinesterase Activity
Iso-valeryl PTZ
1.2
0.8
0.4
0.0
abs/min
-9
-8
-7
-6
-5
[I]
BuChE
AChE

FIG. 6A

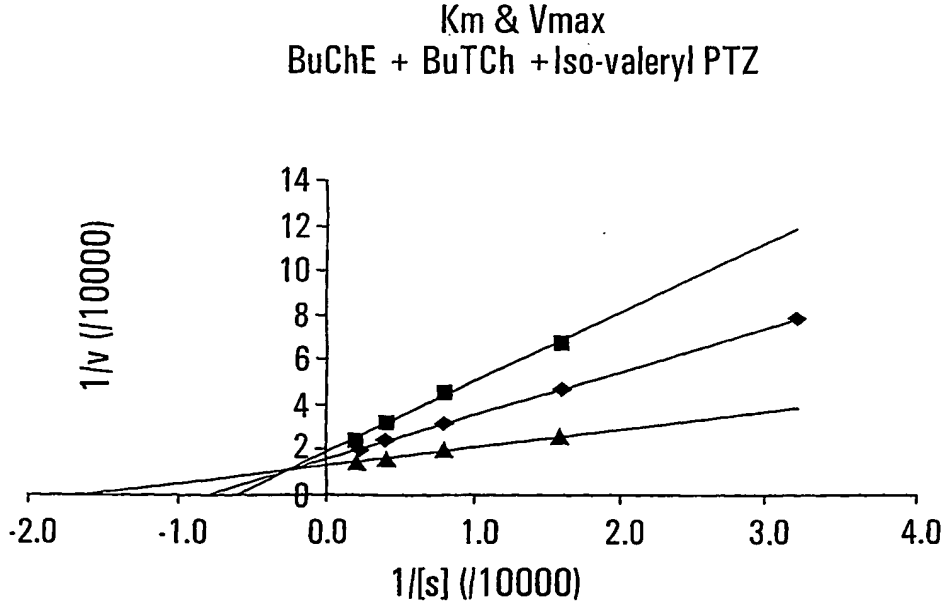

Km & Vmax
BuChE + BuTCh +Iso-valeryl PTZ
14
12
10
8
6
4
2
0
1/v (/10000)
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 6B

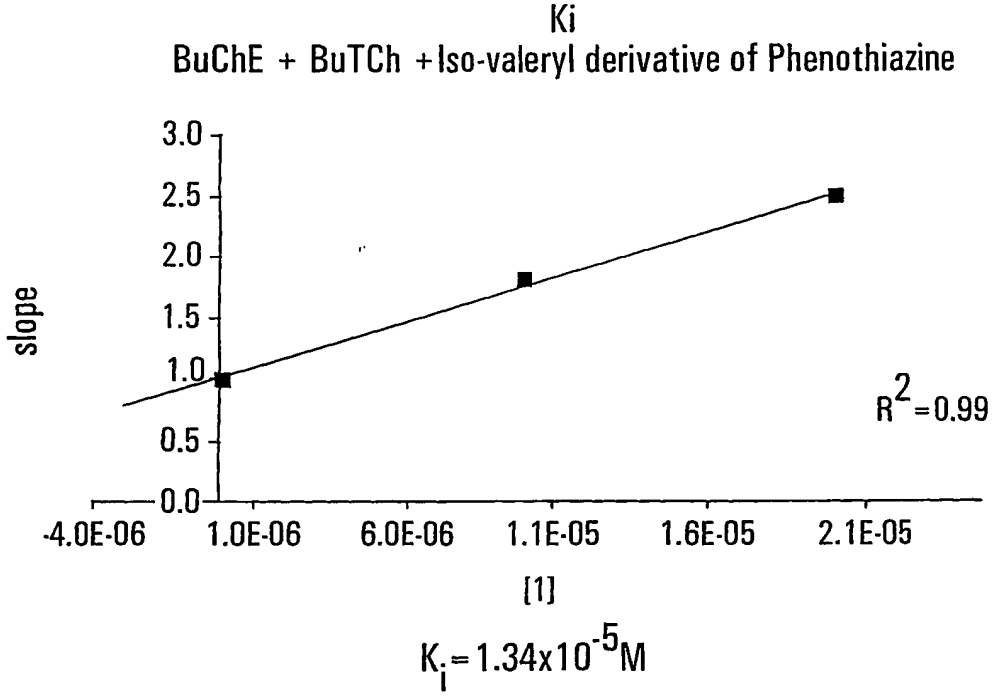

Ki
BuChE + BuTCh + Iso-valeryl derivative of Phenothiazine
slope
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-4.0E-06
1.0E-06
6.0E-06
1.1E-05
1.6E-05
2.1E-05
[I]
R^2=0.99
$K_i = 1.34 \times 10^{-5}$M

FIG. 6C

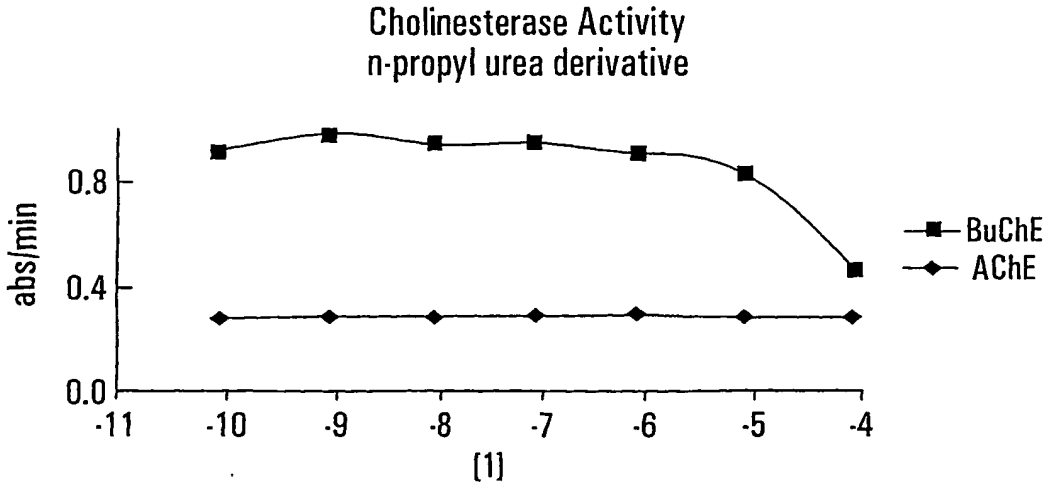

Cholinesterase Activity
n-propyl urea derivative
abs/min
0.8
0.4
0.0
-11
-10
-9
-8
-7
-6
-5
-4
[I]
BuChE
AChE

FIG. 7A

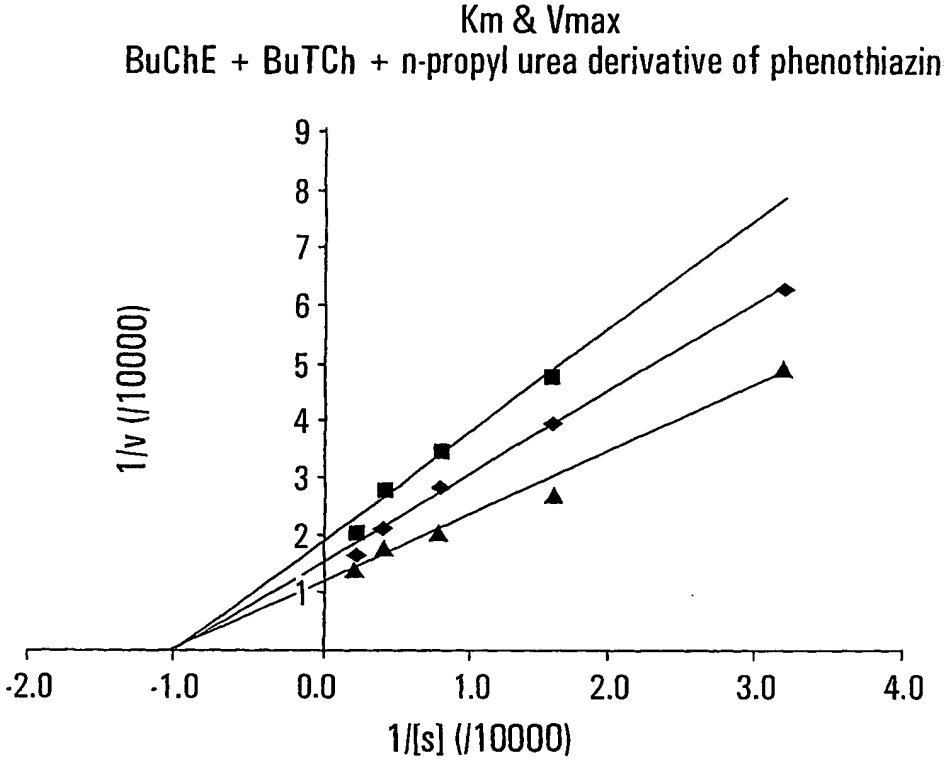

Km & Vmax
BuChE + BuTCh + n-propyl urea derivative of phenothiazine
1/v (/10000)
9
8
7
6
5
4
3
2
1
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 7B

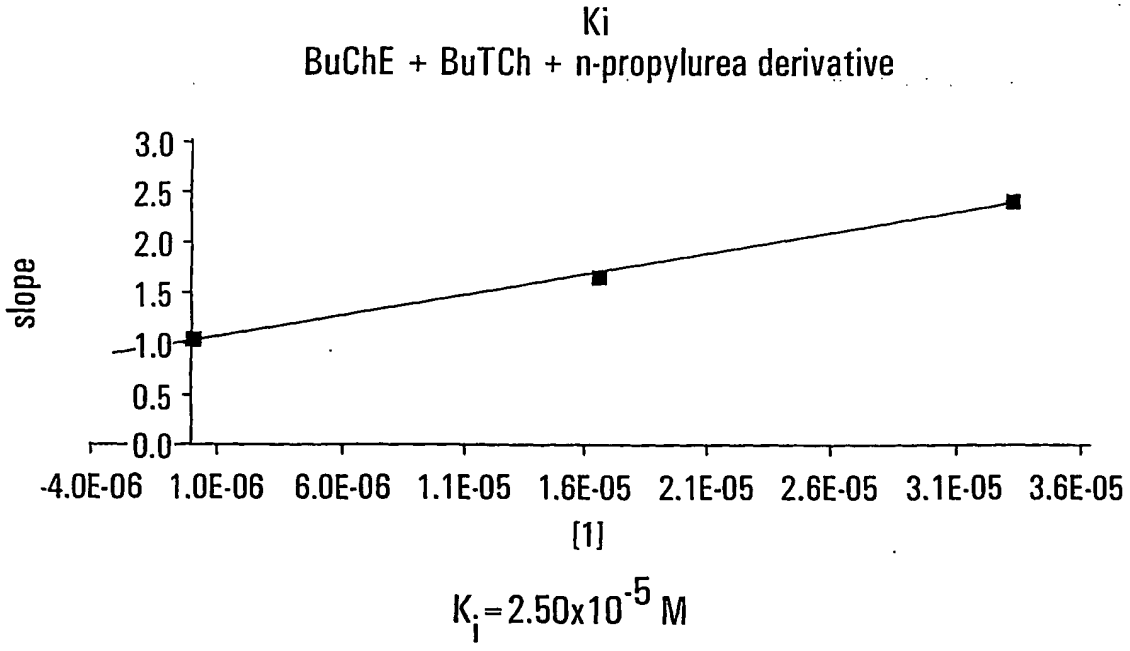

Ki
BuChE + BuTCh + n-propylurea derivative
slope
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-4.0E-06
1.0E-06
6.0E-06
1.1E-05
1.6E-05
2.1E-05
2.6E-05
3.1E-05
3.6E-05
[I]
$K_i = 2.50 \times 10^{-5}$ M

FIG. 7C

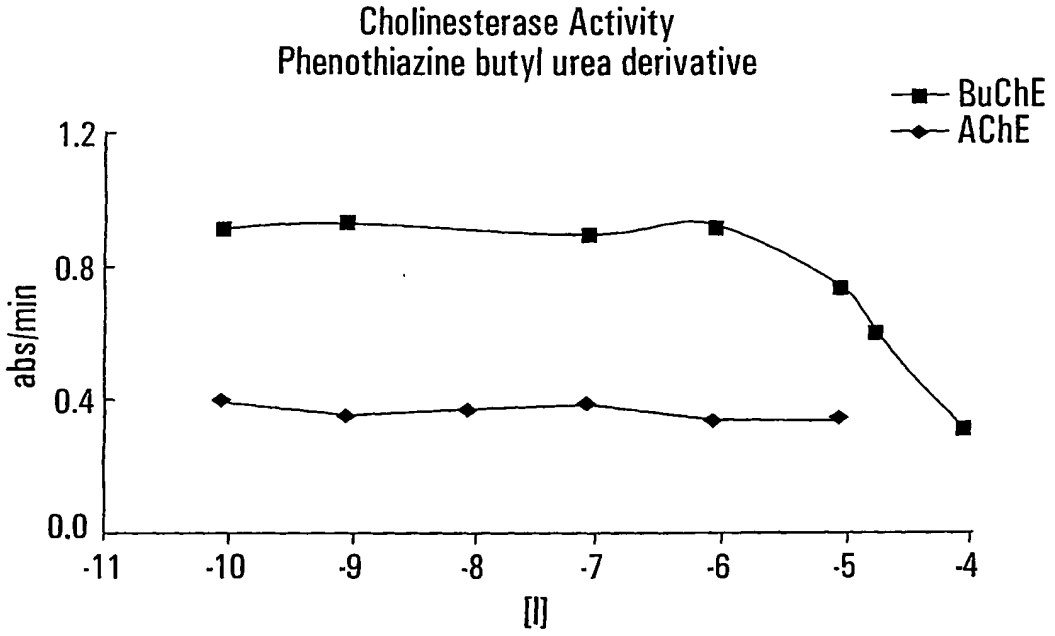

Cholinesterase Activity
Phenothiazine butyl urea derivative
BuChE
AChE
1.2
0.8
0.4
0.0
abs/min
-11
-10
-9
-8
-7
-6
-5
-4
[I]

FIG. 8A

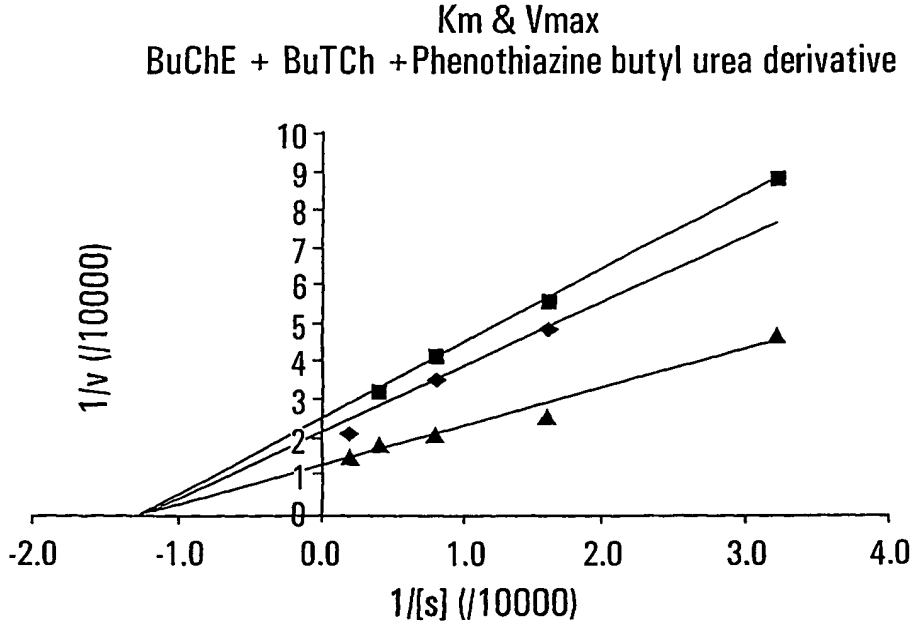

Km & Vmax
BuChE + BuTCh +Phenothiazine butyl urea derivative
10
9
8
7
6
5
4
3
2
1
0
1/v (/10000)
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 8B

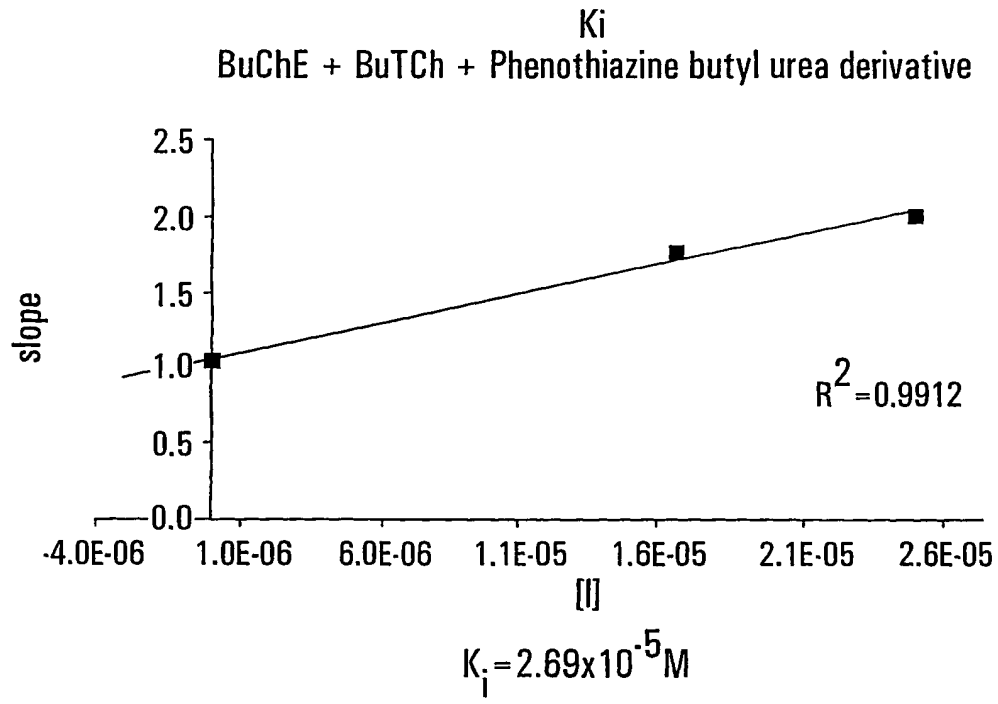

Ki
BuChE + BuTCh + Phenothiazine butyl urea derivative
slope
2.5
2.0
1.5
1.0
0.5
0.0
R^2=0.9912
-4.0E-06
1.0E-06
6.0E-06
1.1E-05
1.6E-05
2.1E-05
2.6E-05
[I]
$K_i = 2.69x10^{-5}M$

FIG. 8C

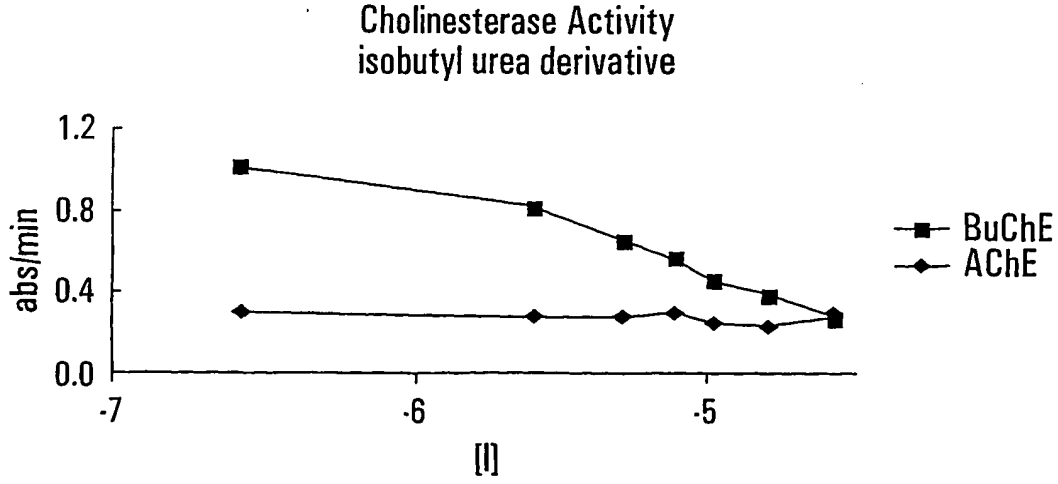

Cholinesterase Activity
isobutyl urea derivative
abs/min
1.2
0.8
0.4
0.0
-7
-6
-5
[I]
BuChE
AChE

FIG. 9A

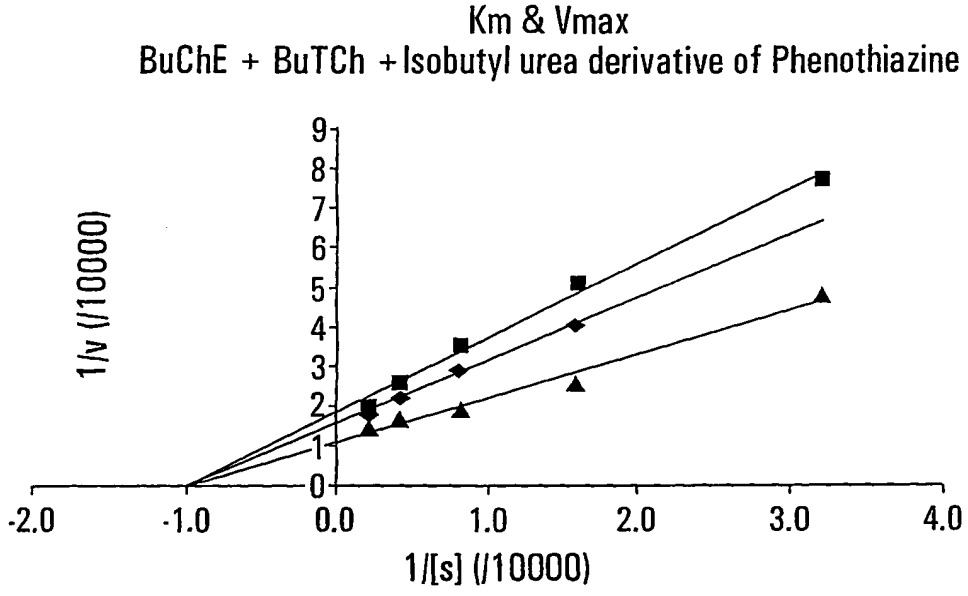

Km & Vmax
BuChE + BuTCh + Isobutyl urea derivative of Phenothiazine
1/v (/10000)
9
8
7
6
5
4
3
2
1
0
-2.0
-1.0
0.0
1.0
2.0
3.0
4.0
1/[s] (/10000)

FIG. 9B

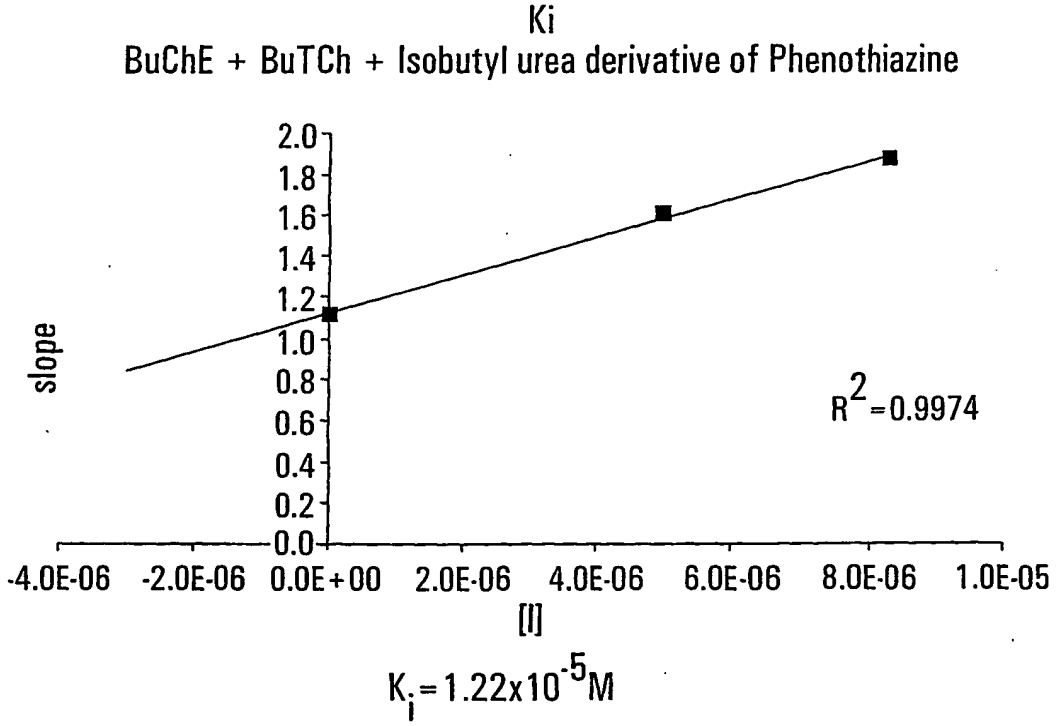

Ki
BuChE + BuTCh + Isobutyl urea derivative of Phenothiazine
slope
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-4.0E-06
-2.0E-06
0.0E+00
2.0E-06
4.0E-06
6.0E-06
8.0E-06
1.0E-05
[I]
R2=0.9974
Ki=1.22x10-5M

FIG. 9C

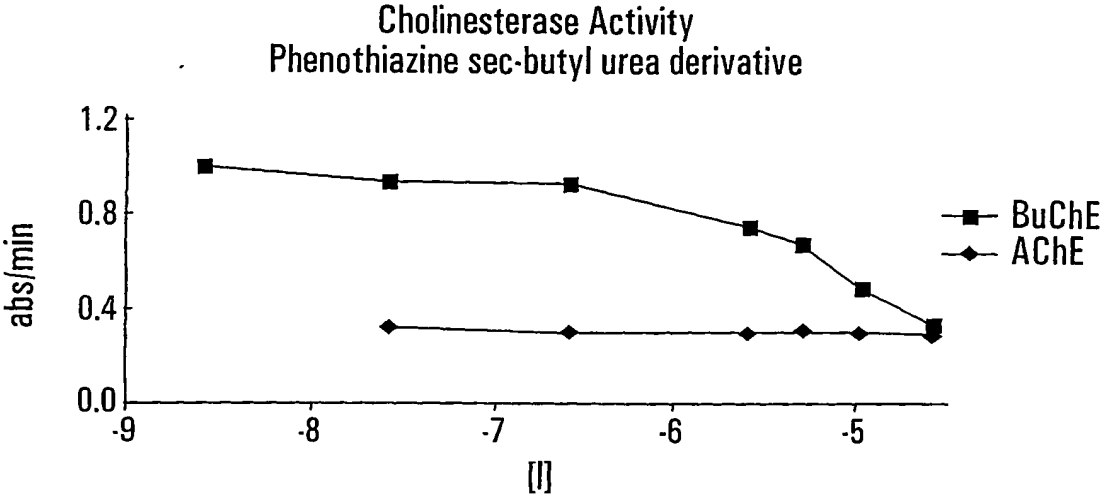


FIG. 10A

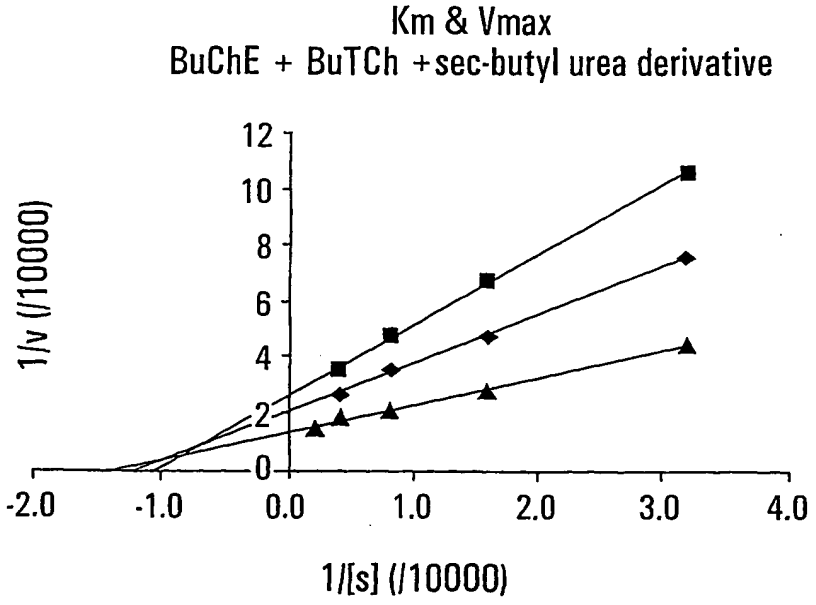


FIG. 10B

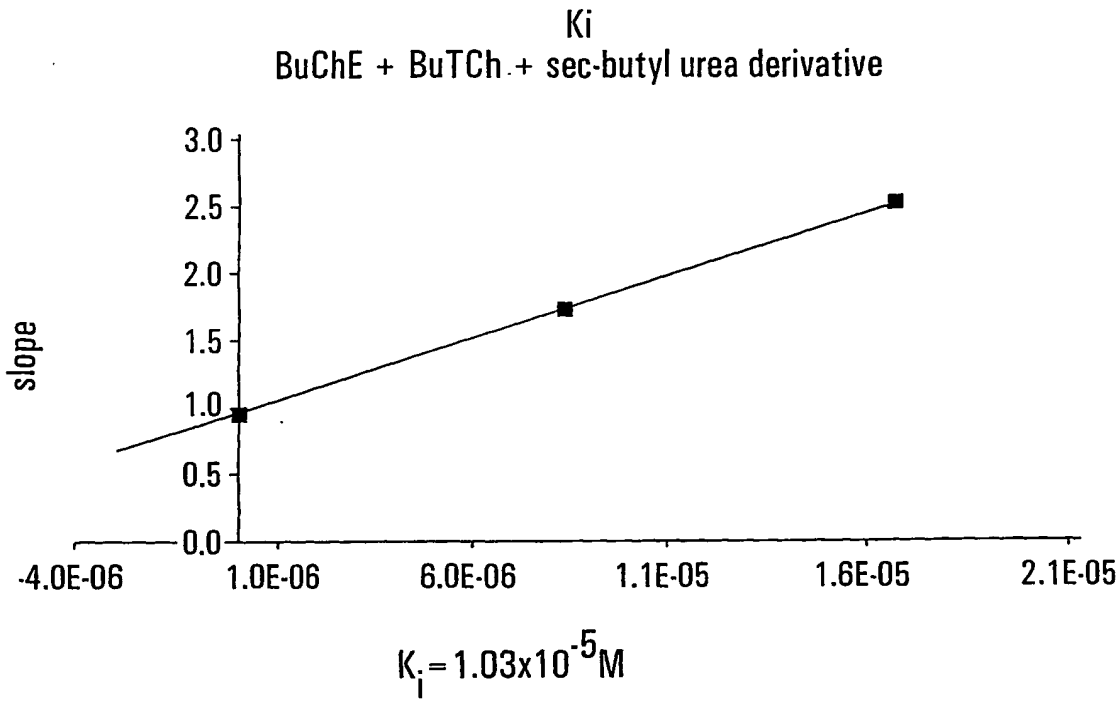


FIG. 10C

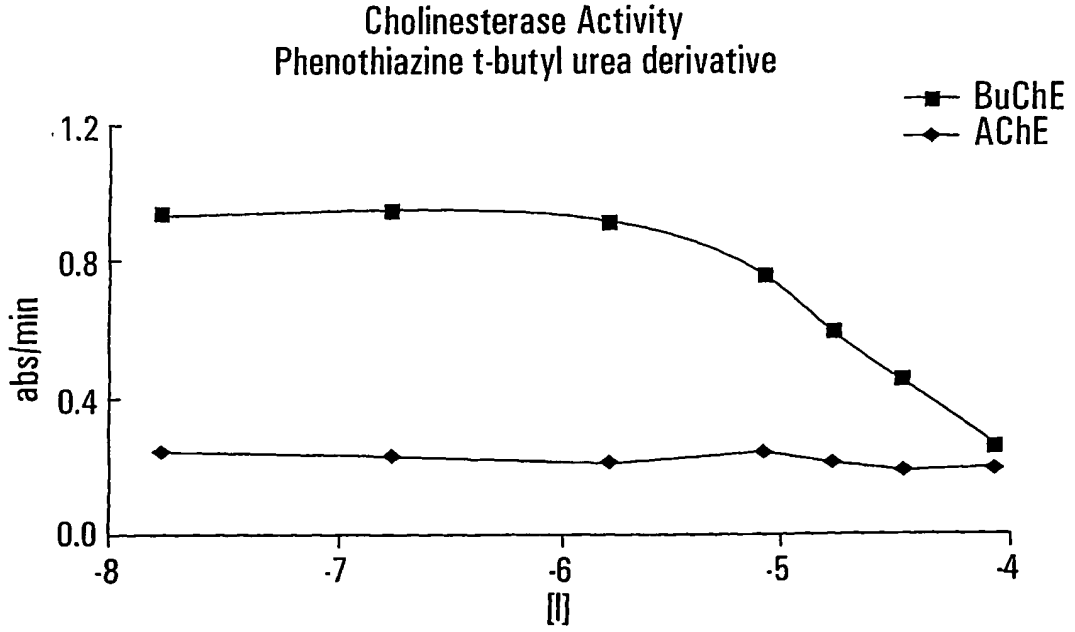


FIG. 11A

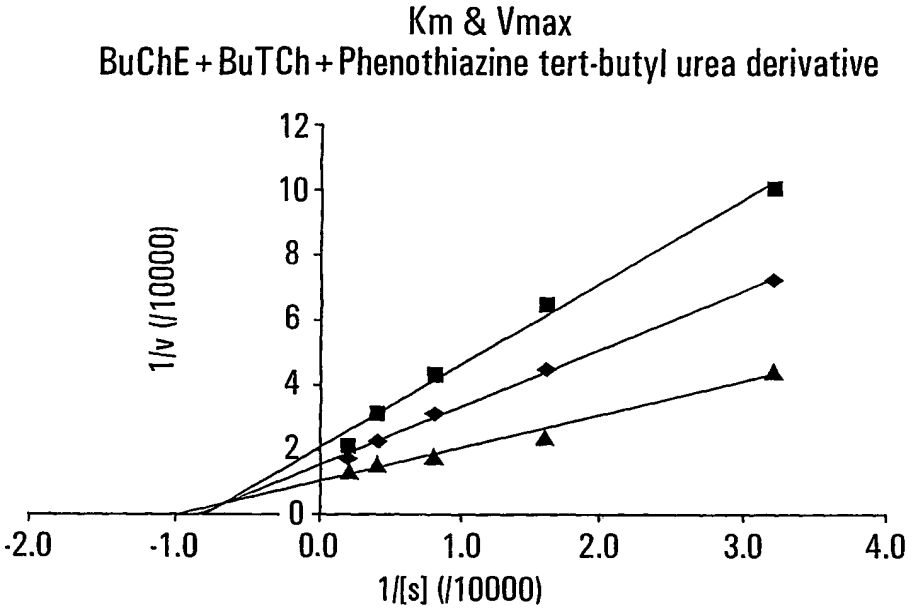


FIG. 11B

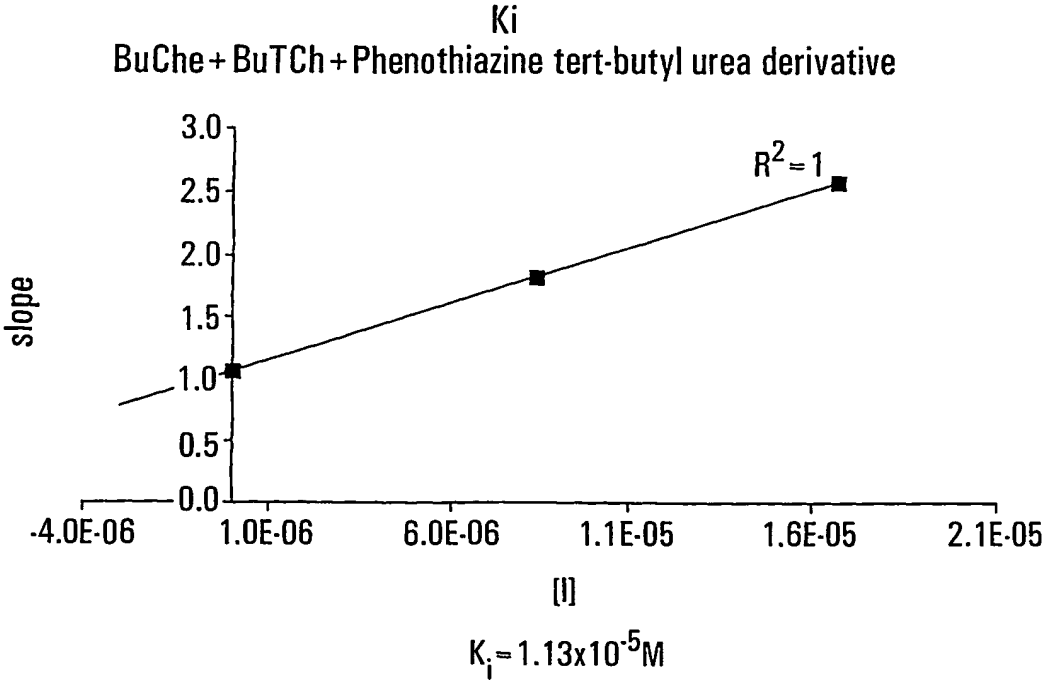


FIG. 11C

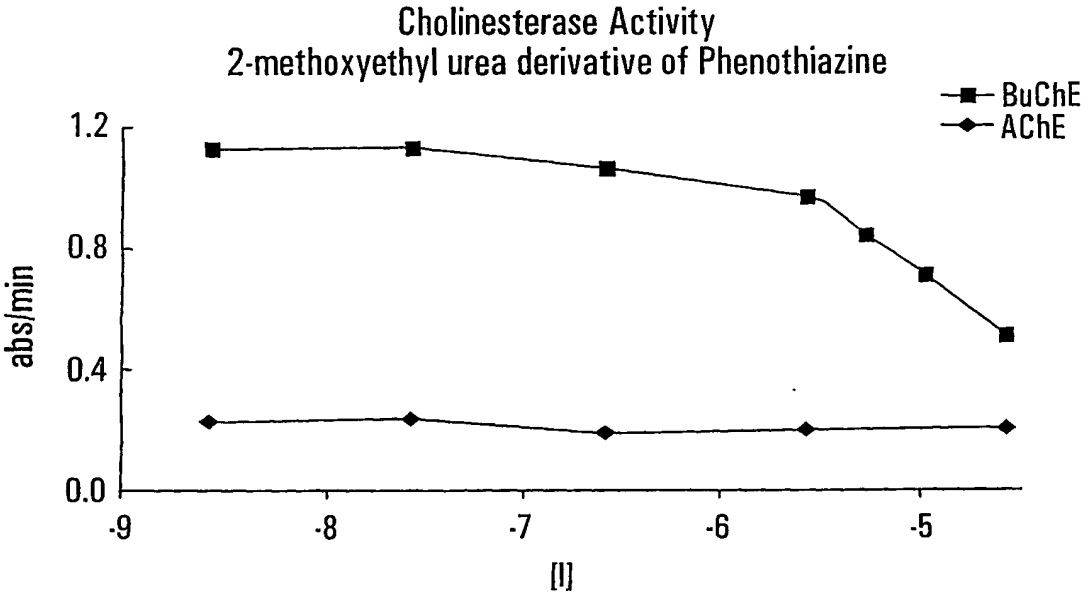


FIG. 12A

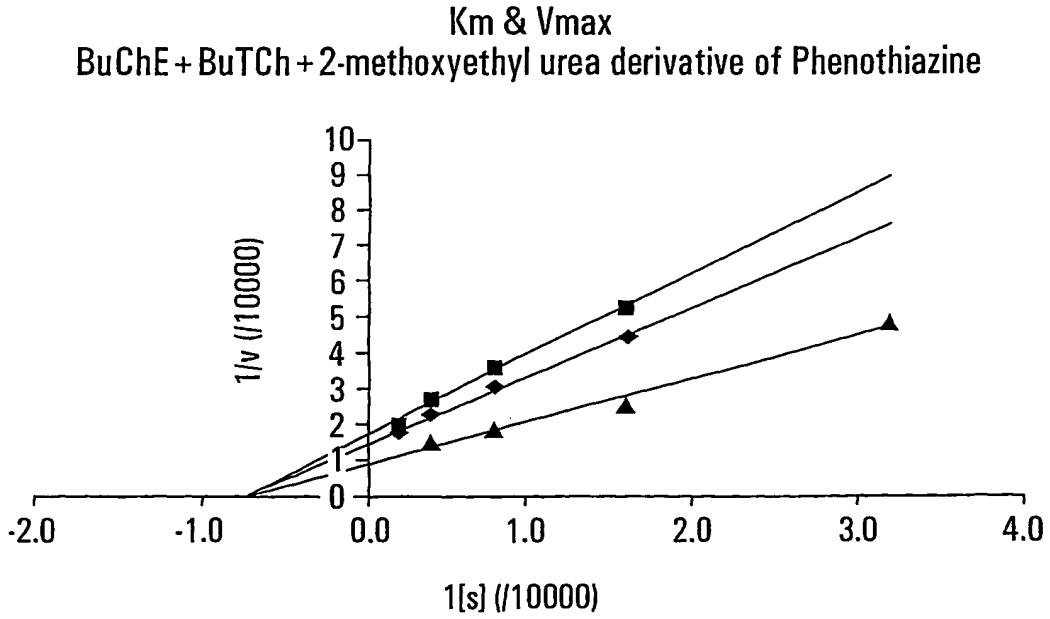


FIG. 12B

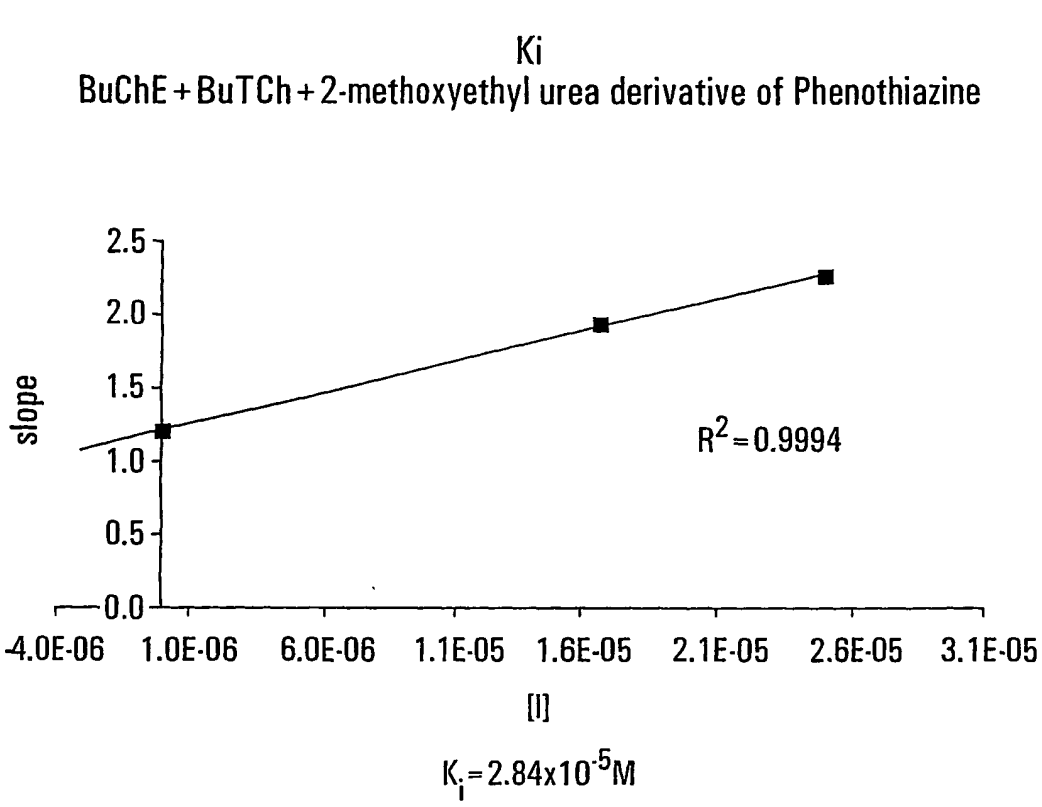


FIG. 12C

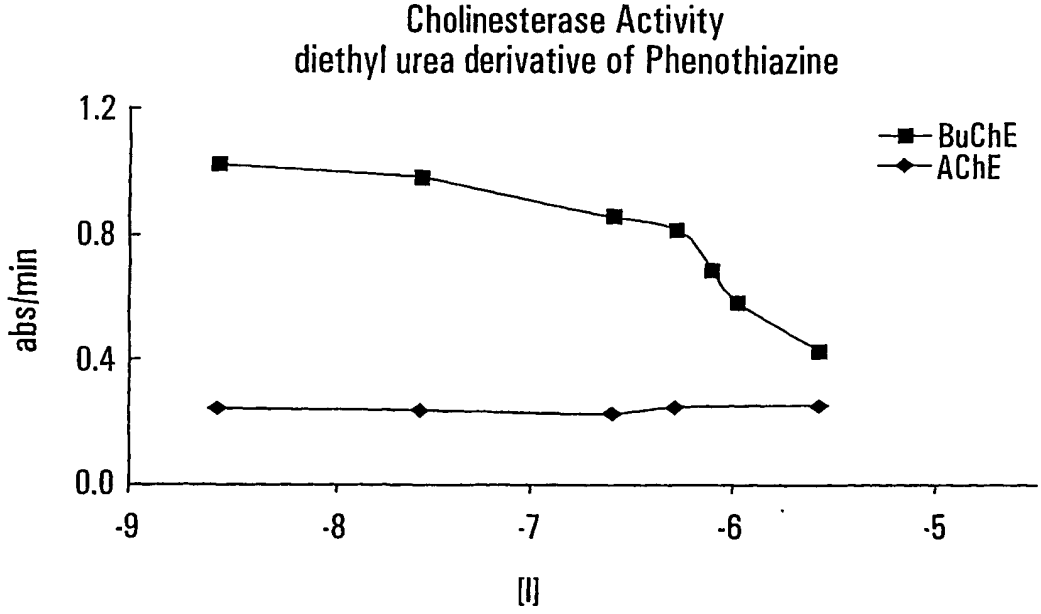


FIG. 13A

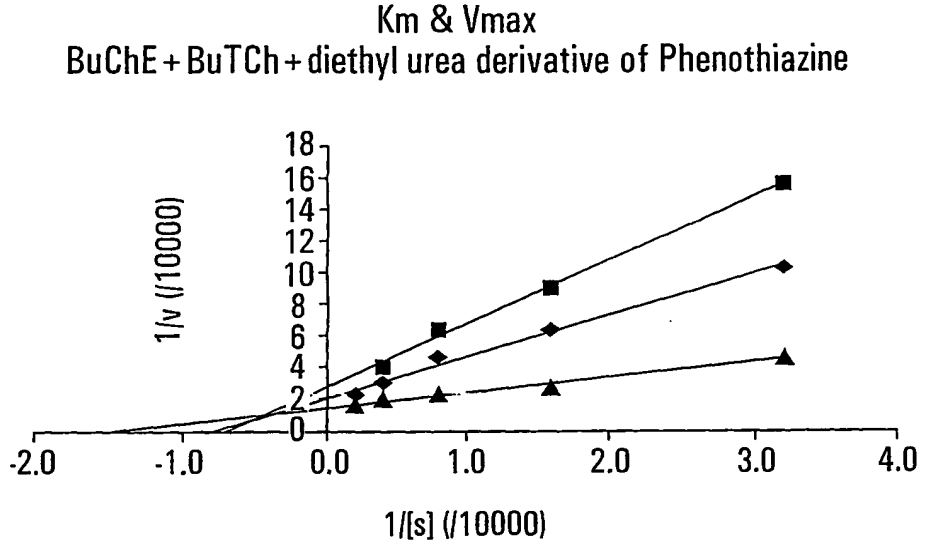


FIG. 13B

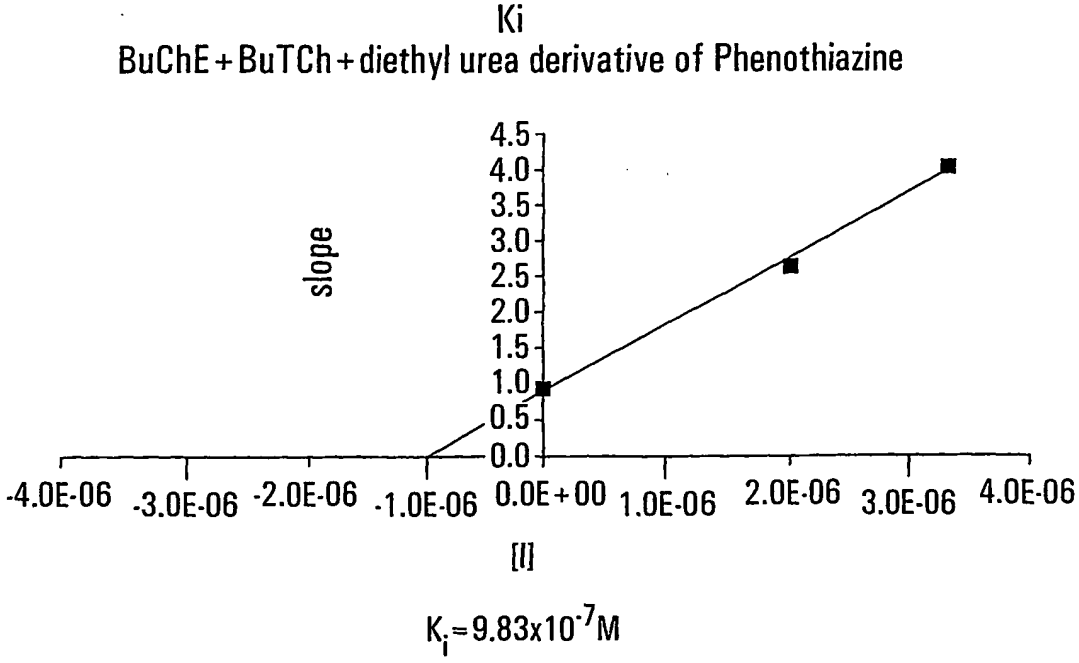


FIG. 13C

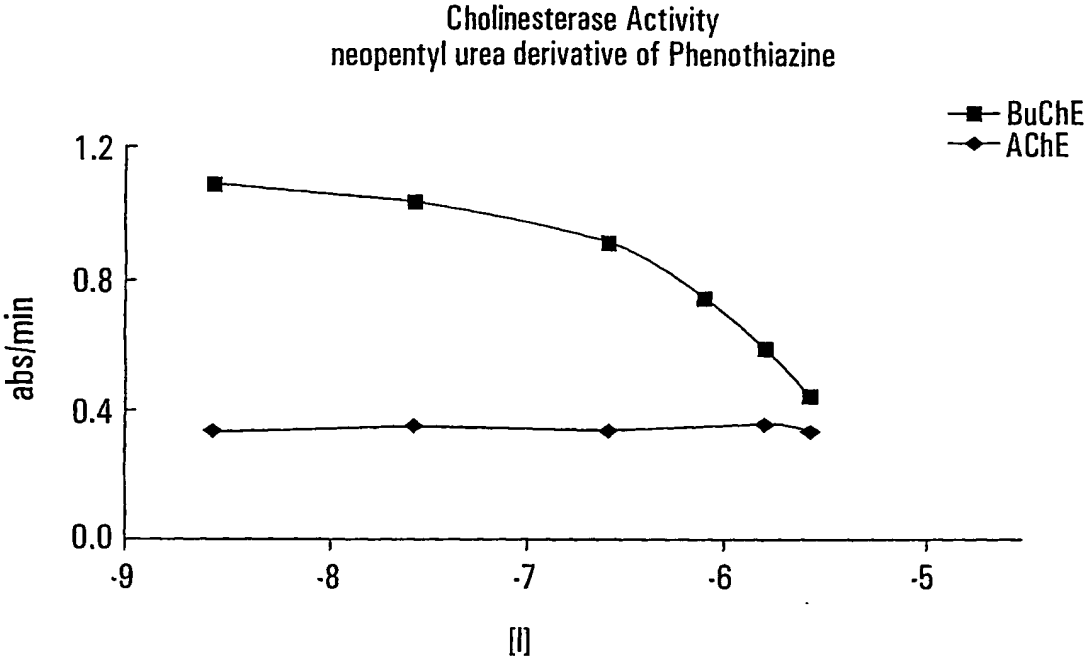


FIG. 14A

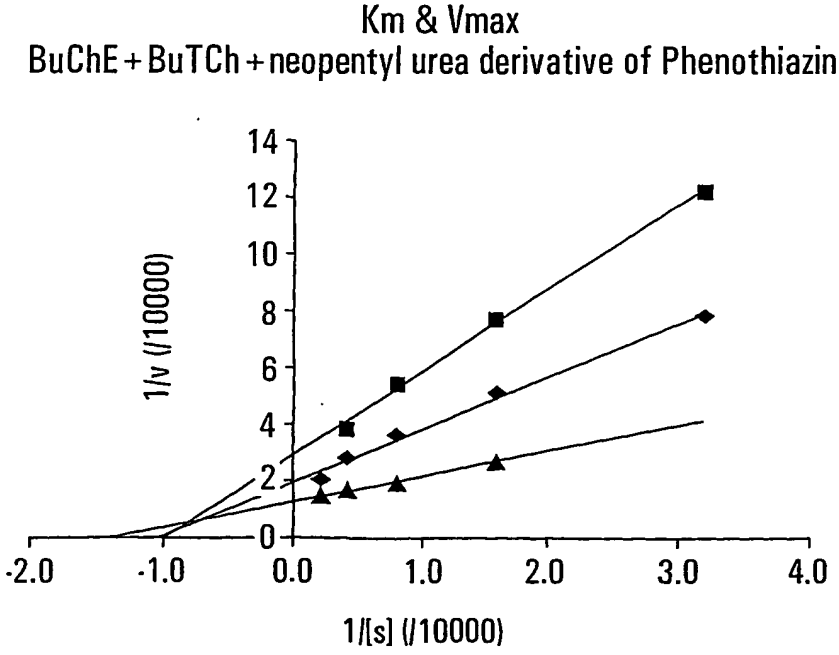


FIG. 14B

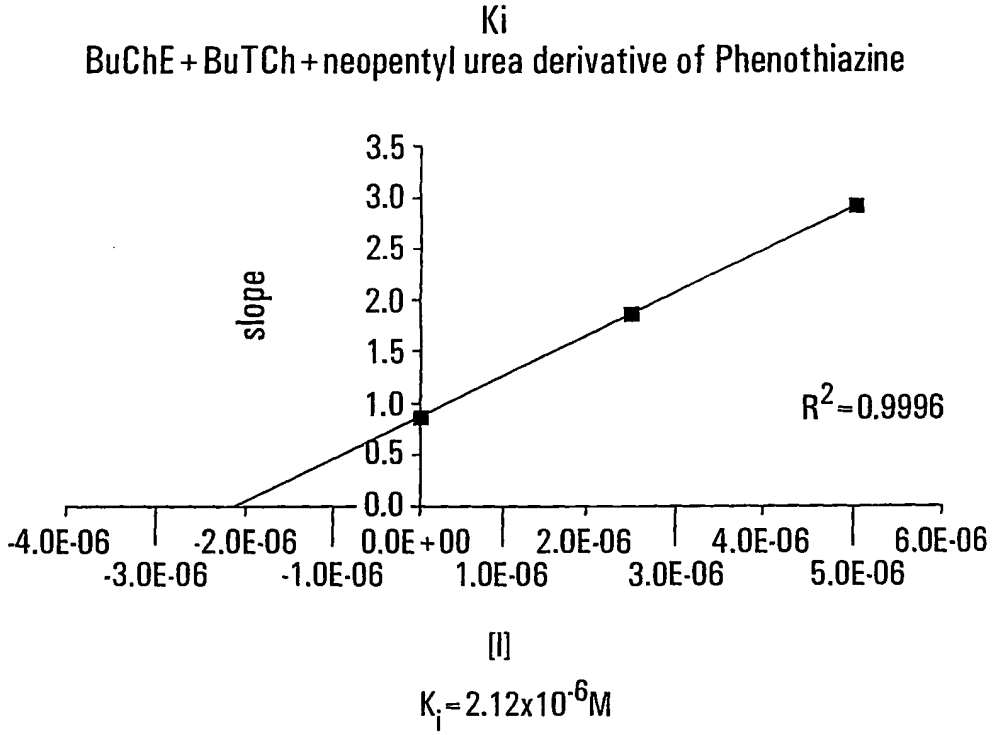


FIG. 14C

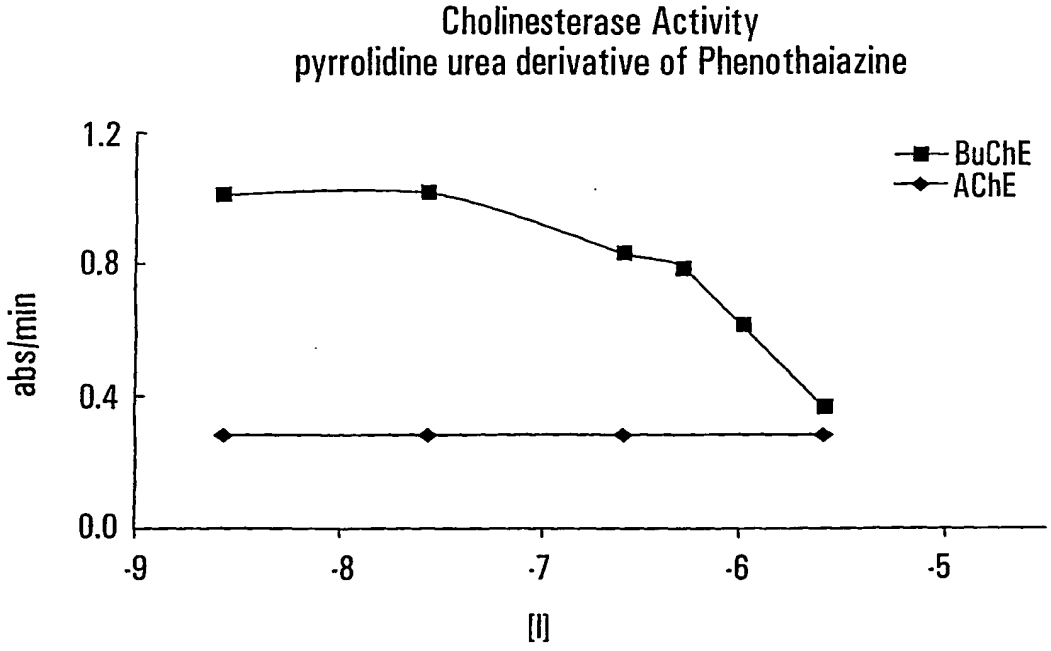


FIG. 15A

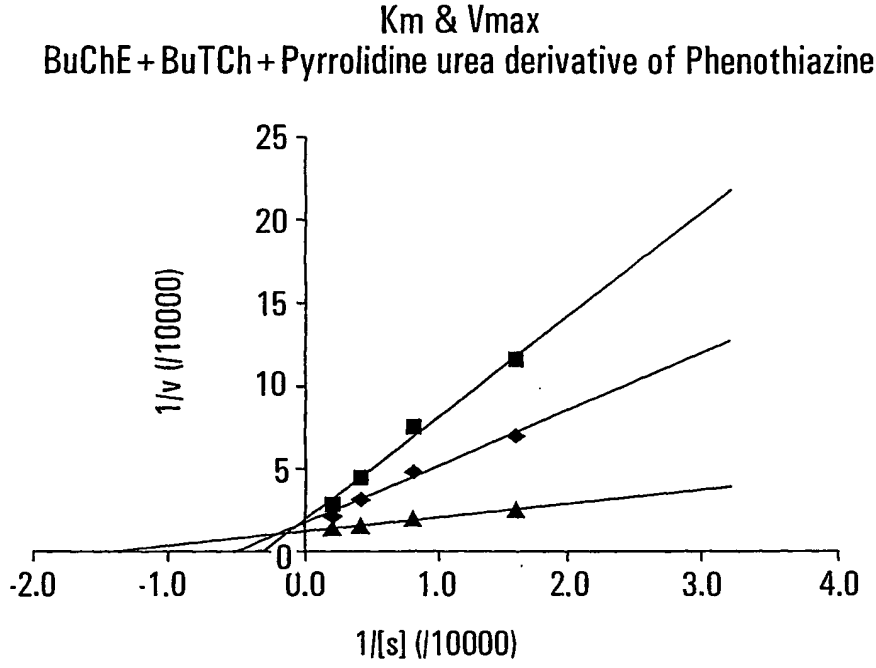


FIG. 15B

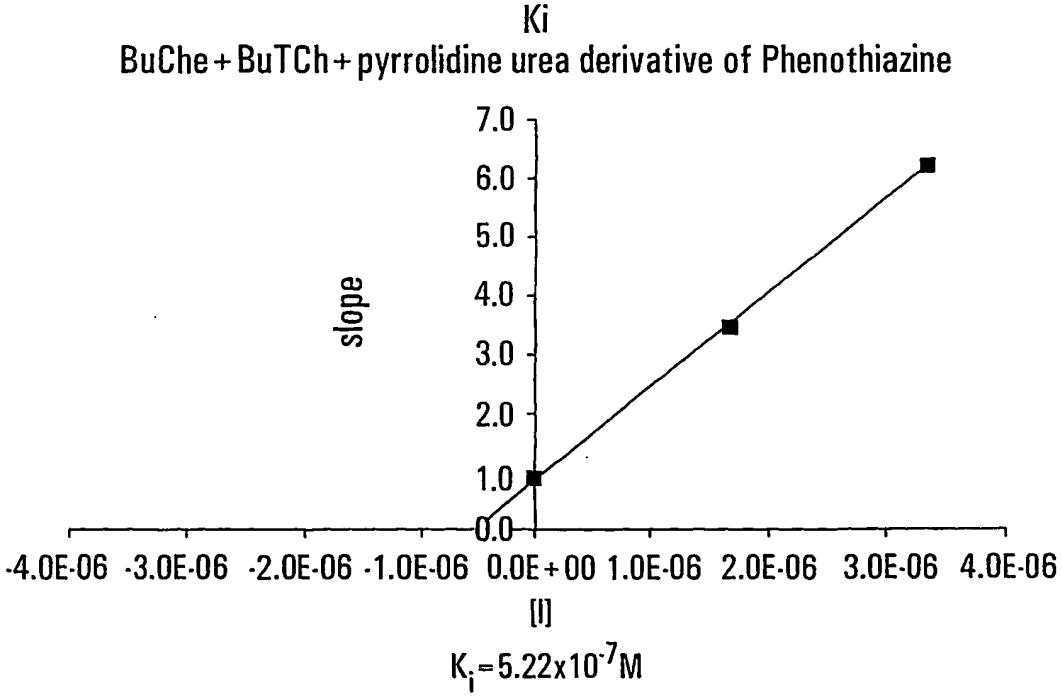


FIG. 15C

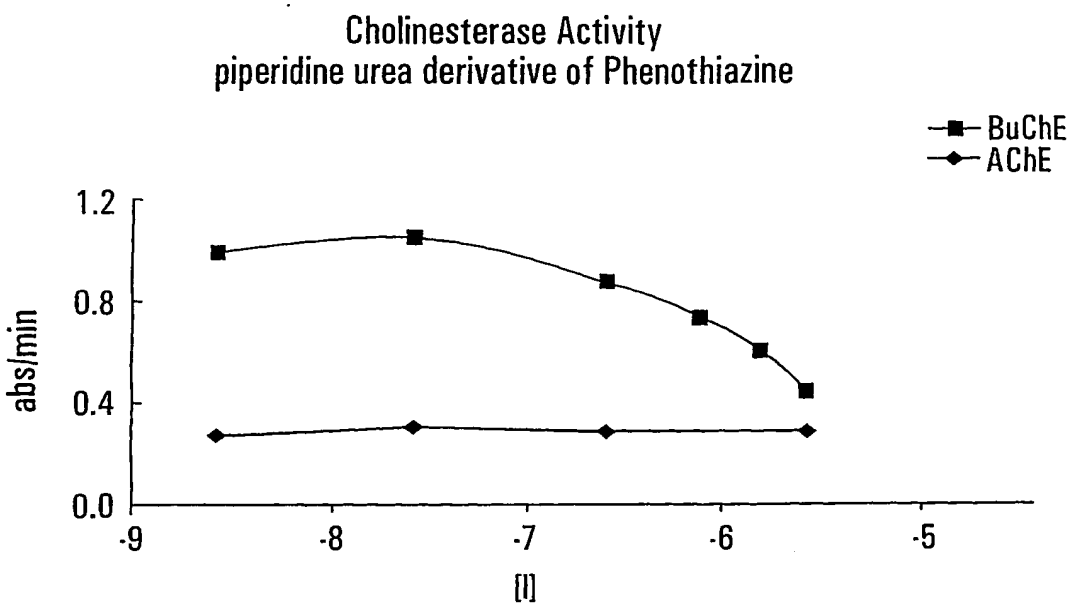


FIG. 16A

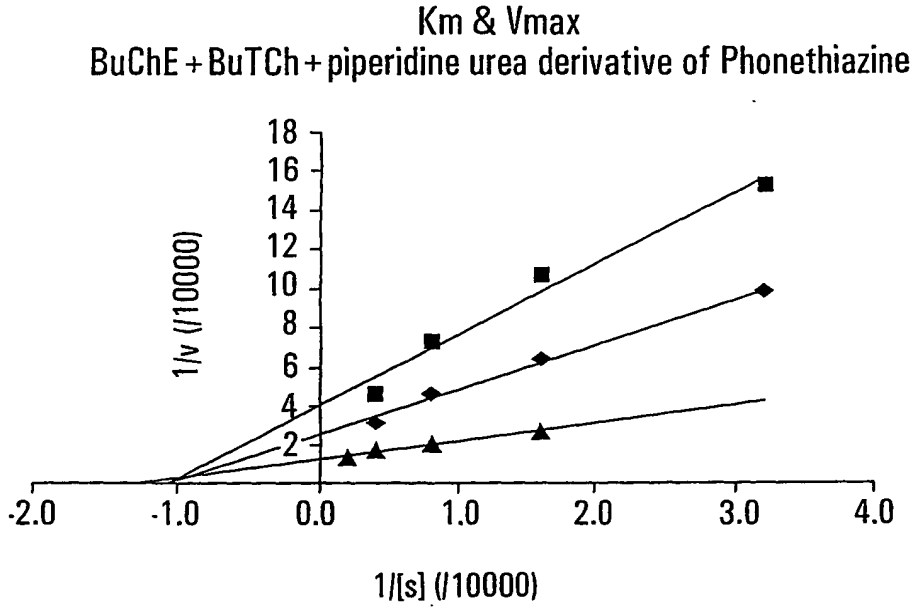


FIG. 16B

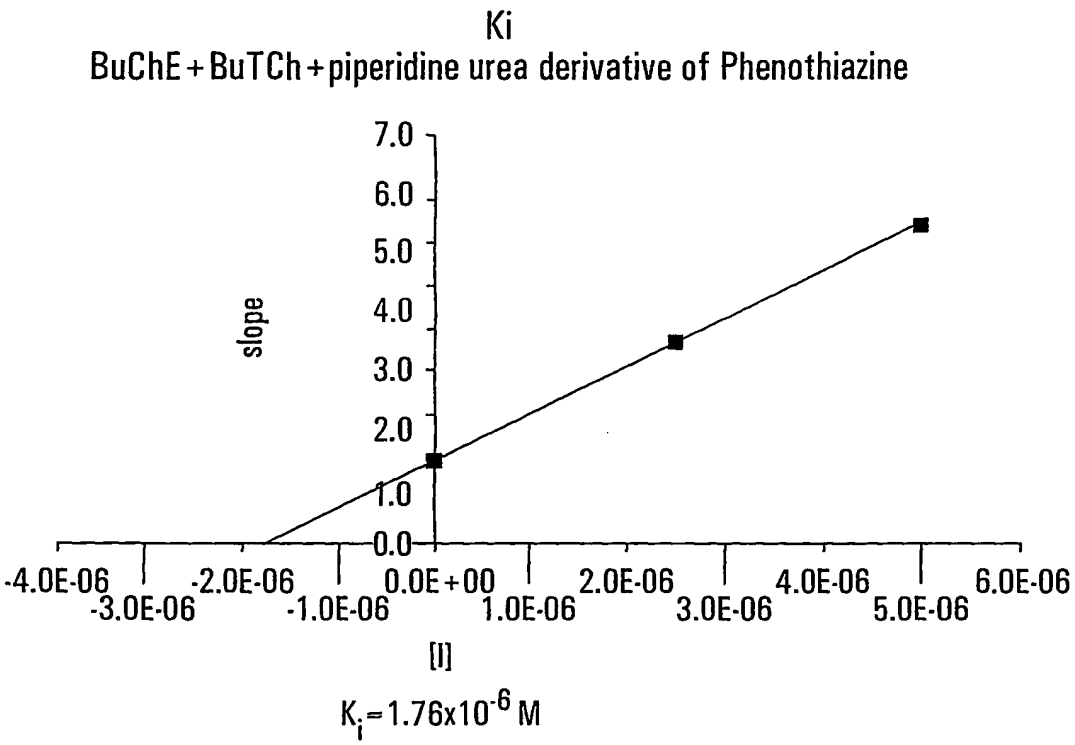


FIG. 16C

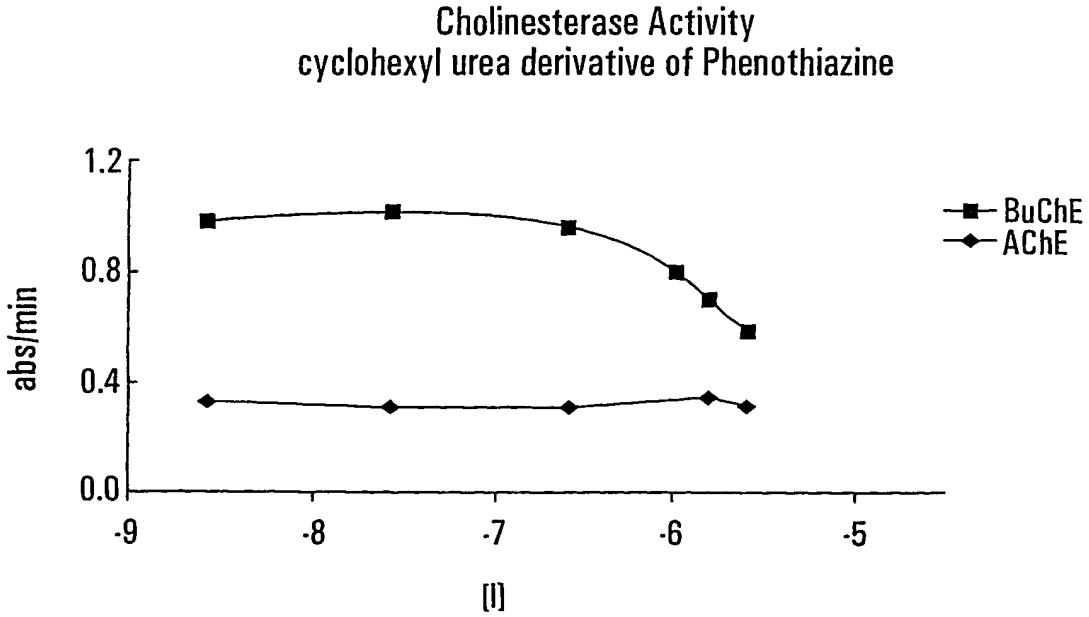


FIG. 17A

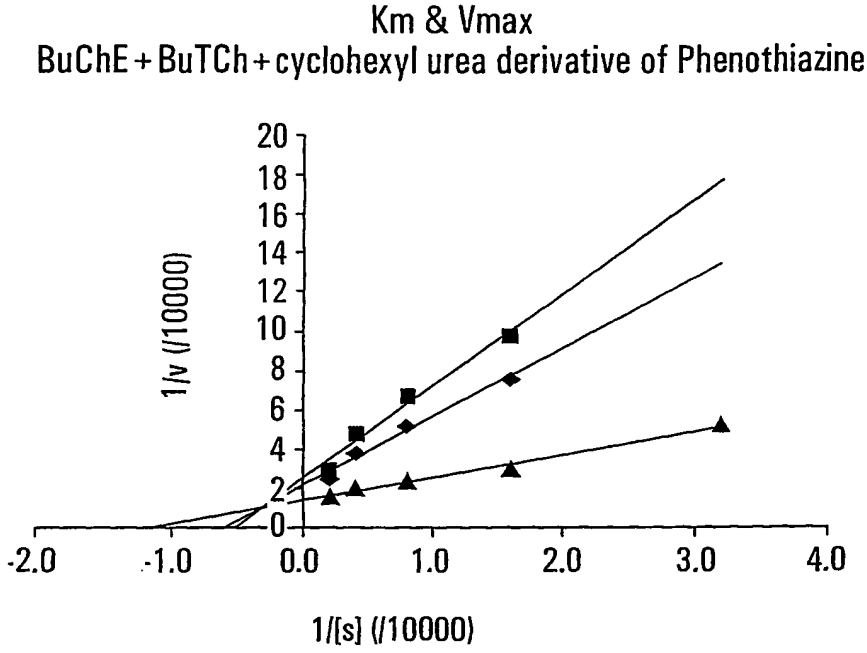


FIG. 17B

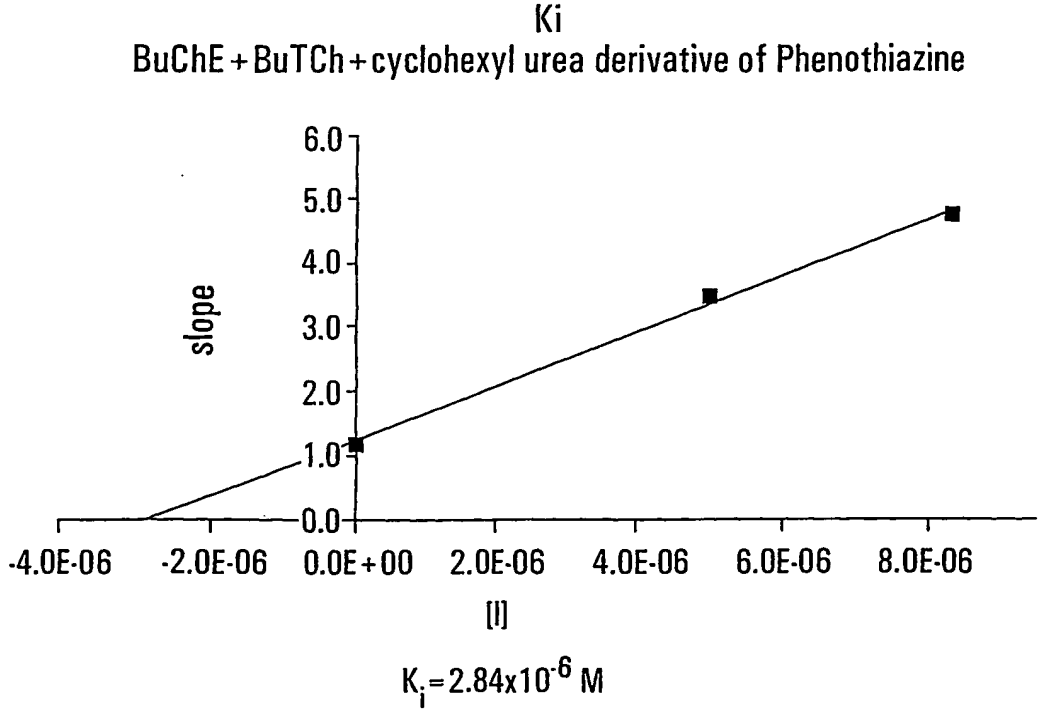


FIG. 17C

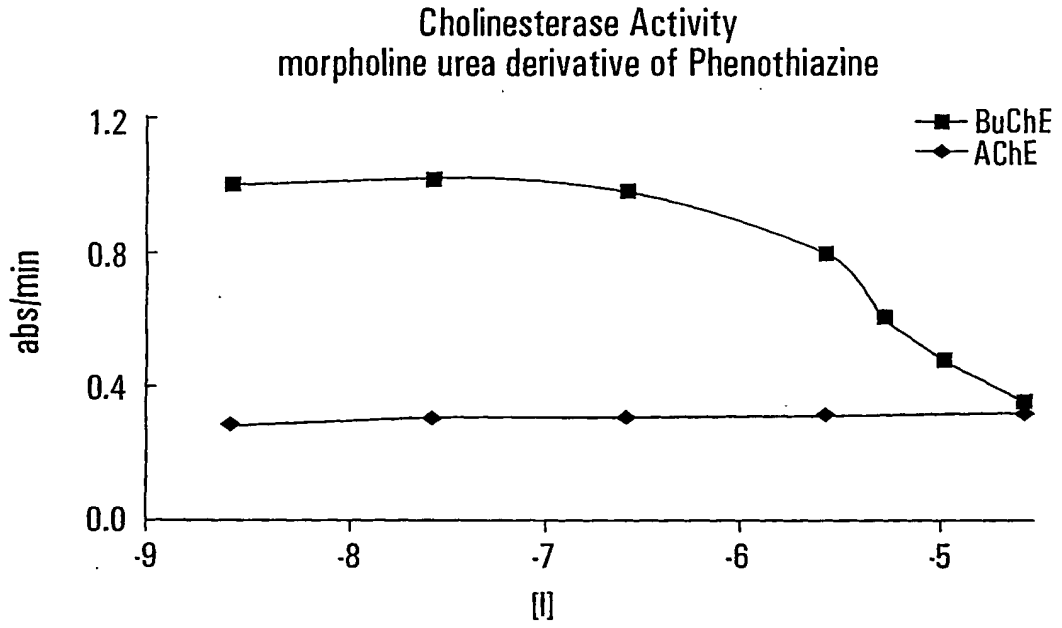


FIG. 18A

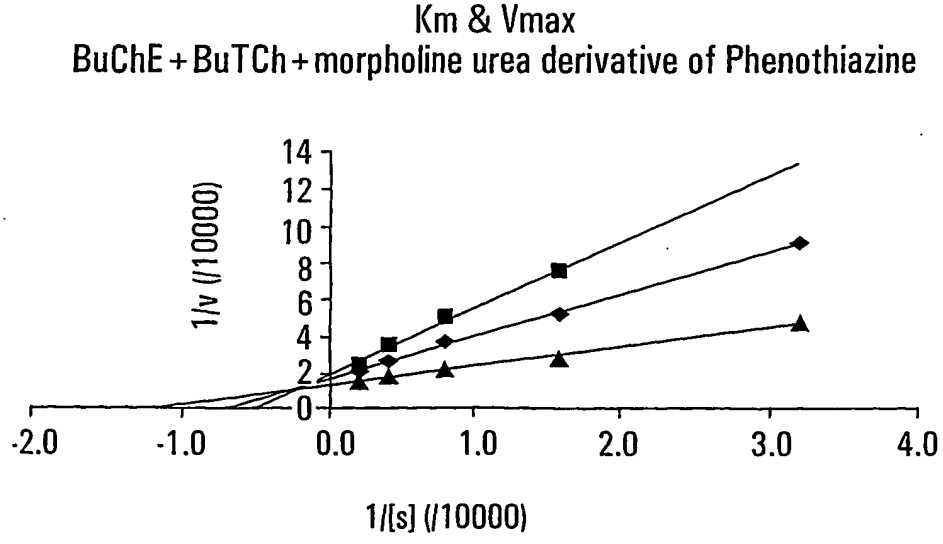


FIG. 18B

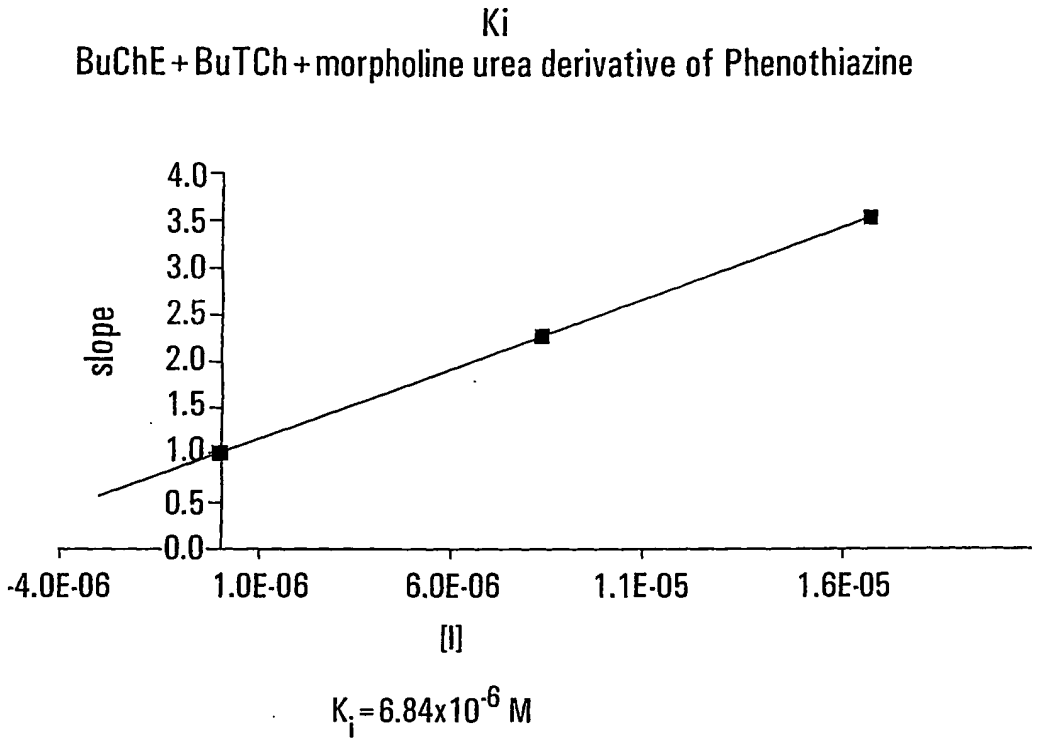


FIG. 18C

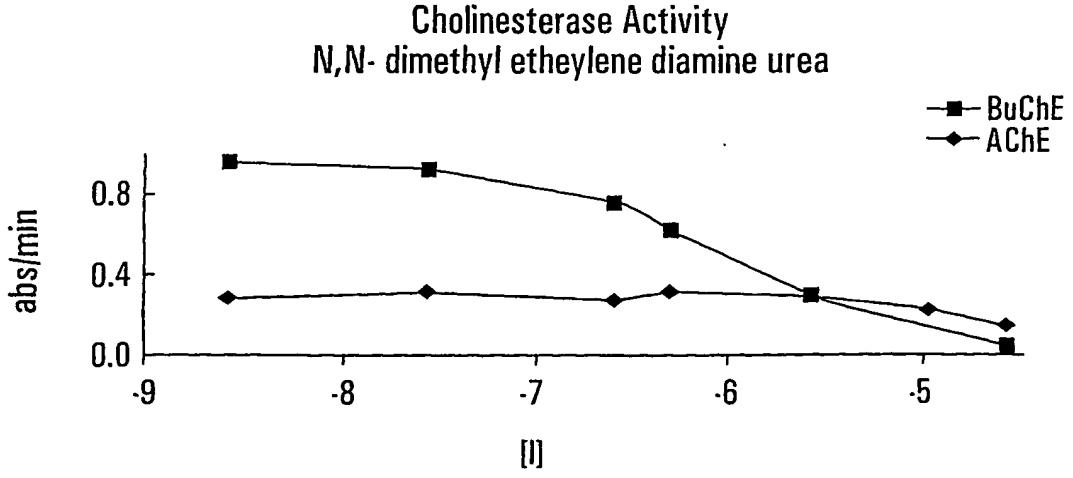


FIG. 19A

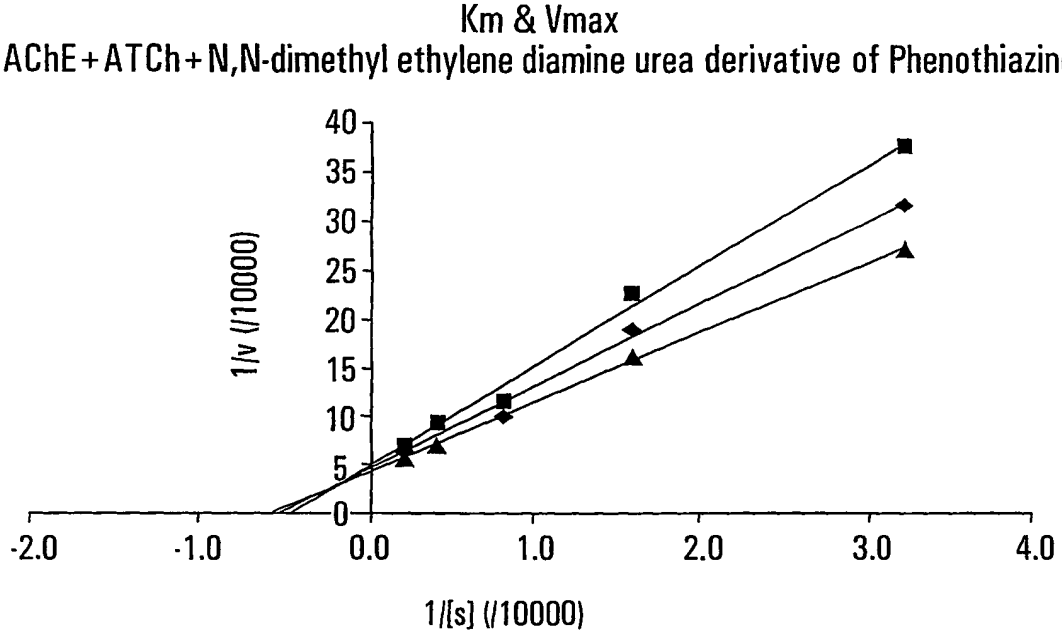


FIG. 19B

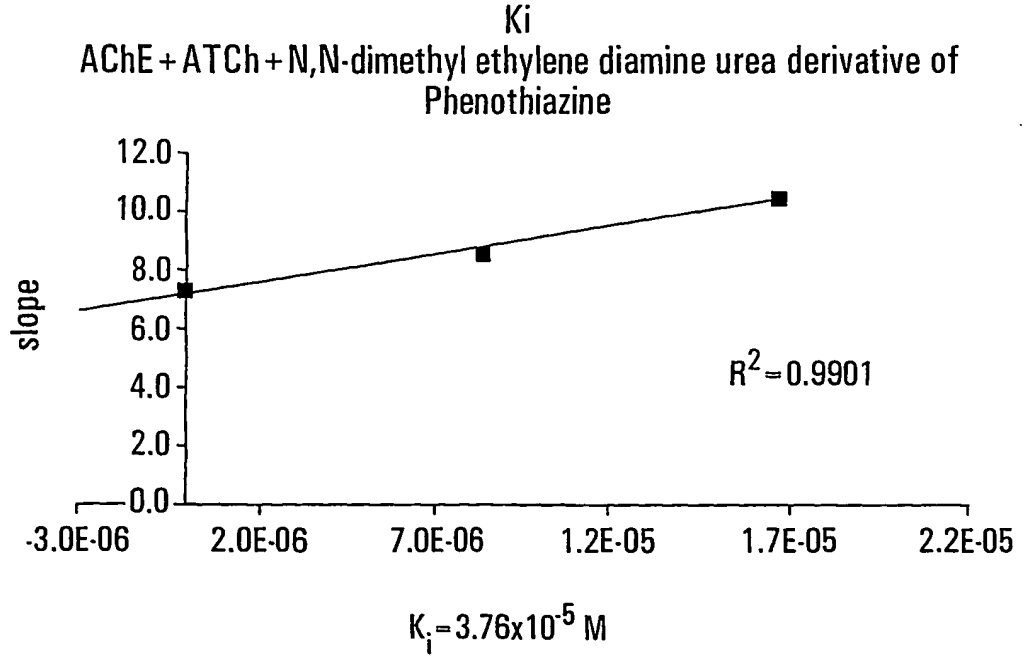


FIG. 19C

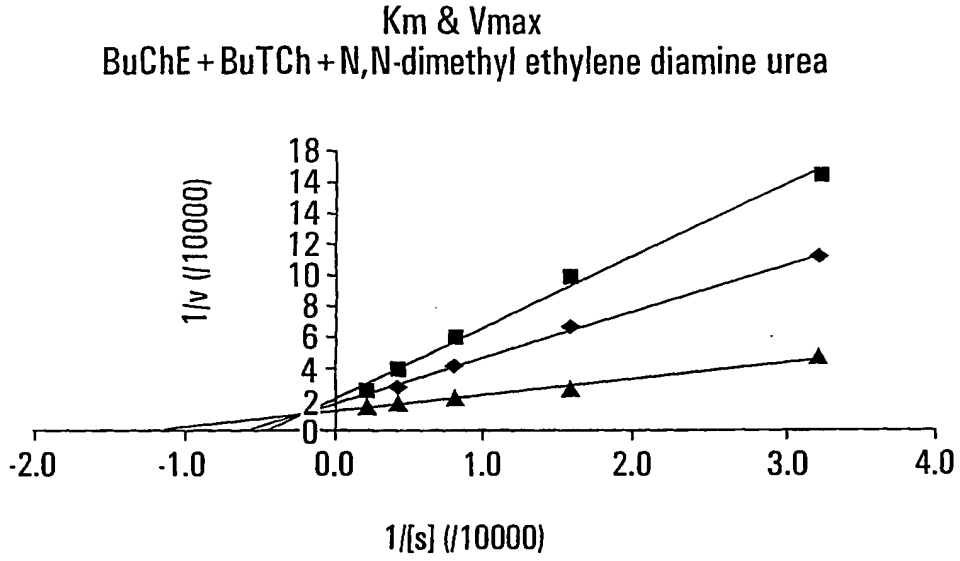


FIG. 19D

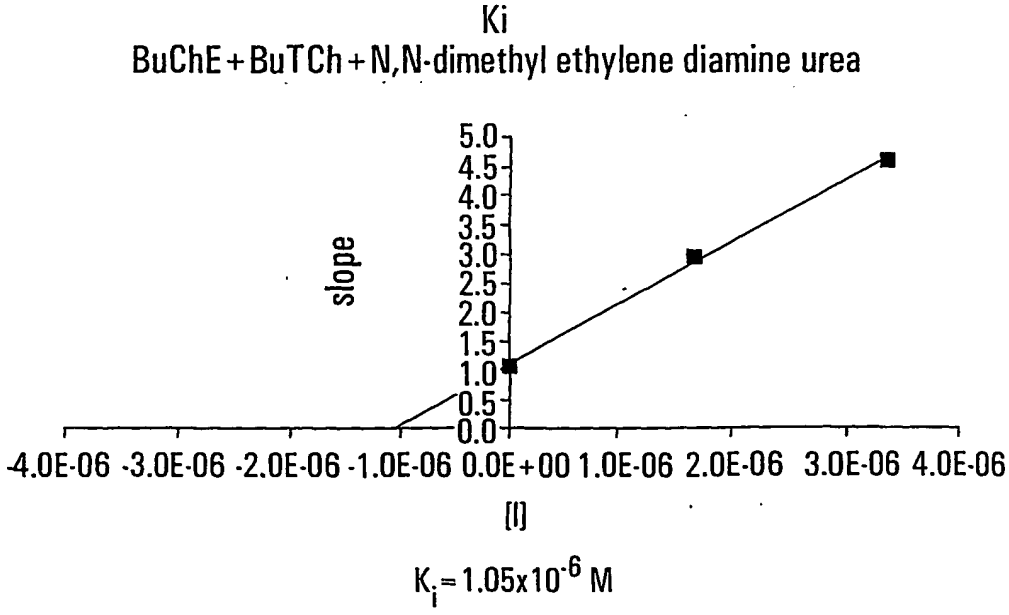


FIG. 19E

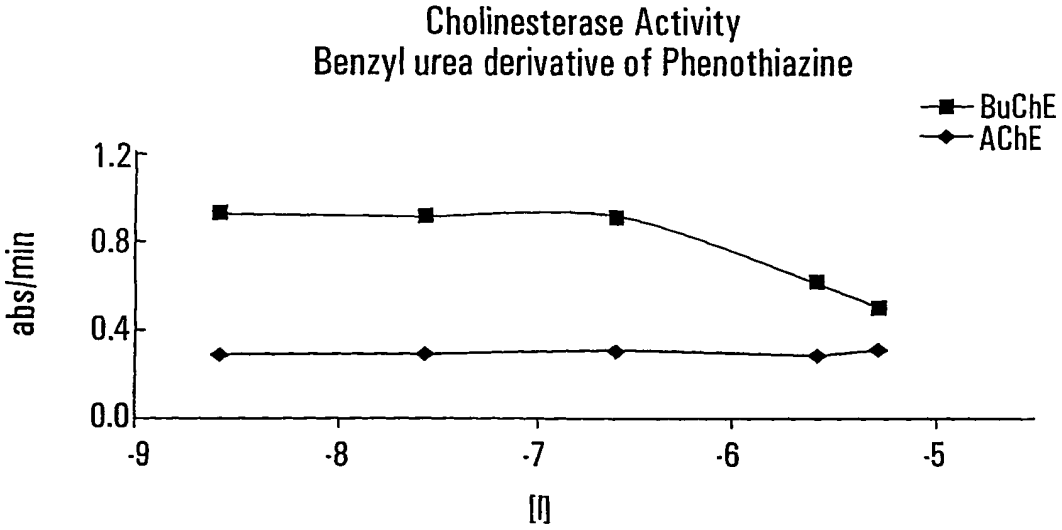


FIG. 20A

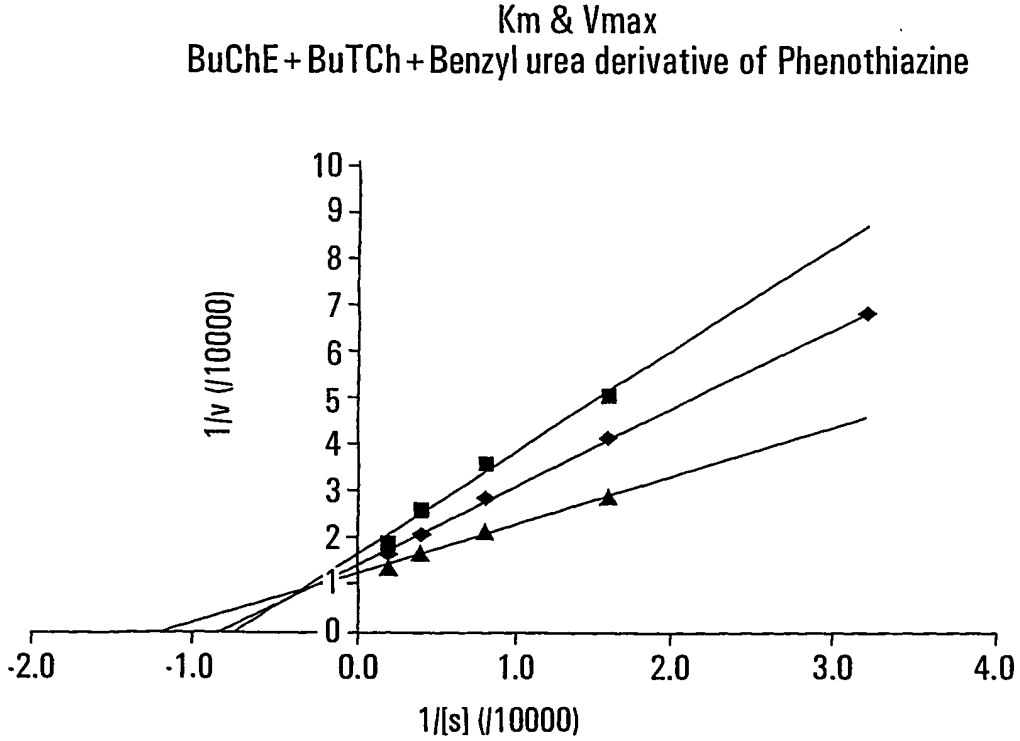


FIG. 20B

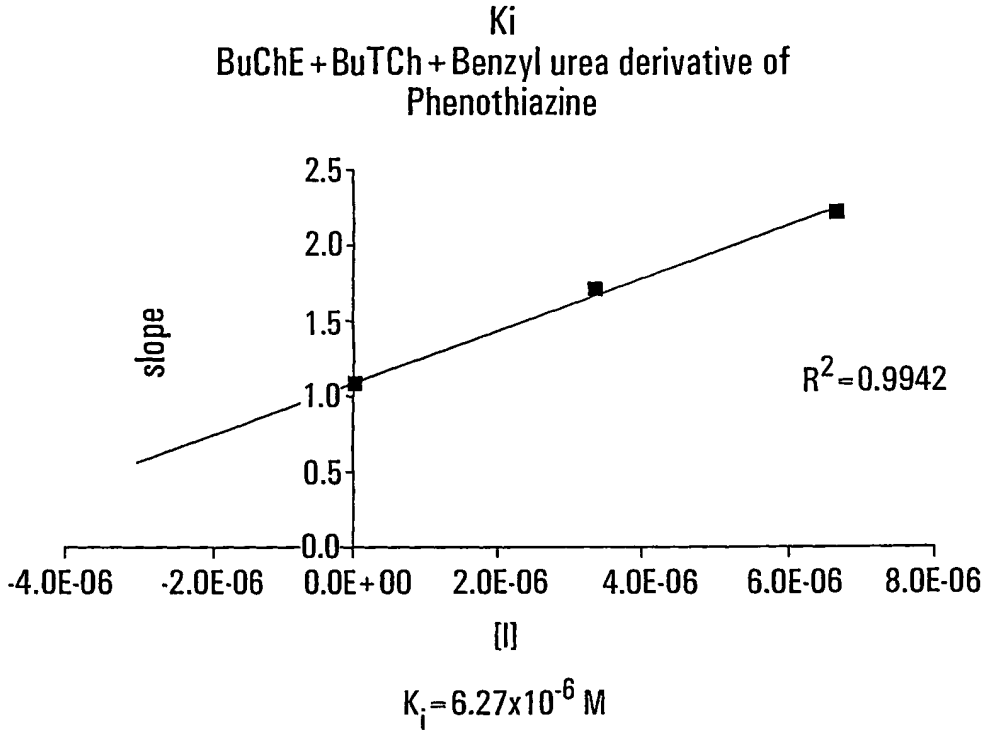


FIG. 20C

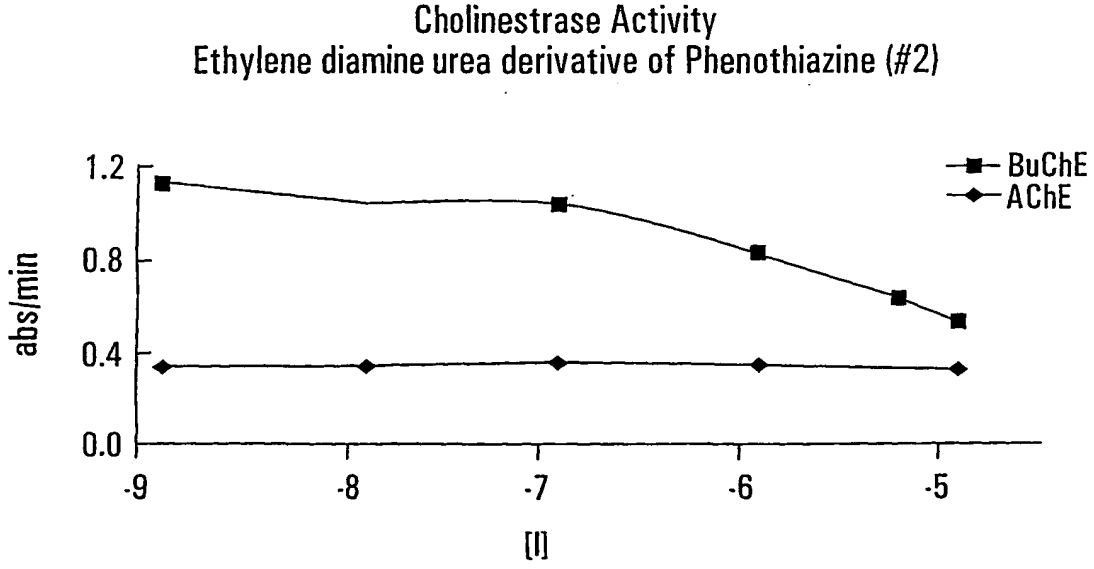


FIG. 21A

Km & Vmax
BuChE + BuTCh + Ethylene diamine urea derivative of Phenothiazine

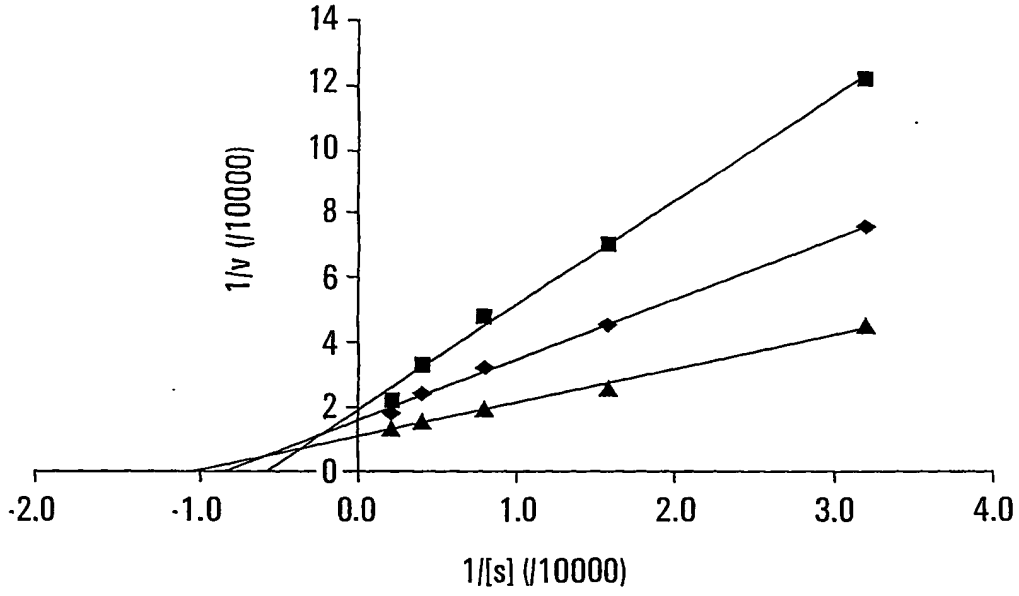
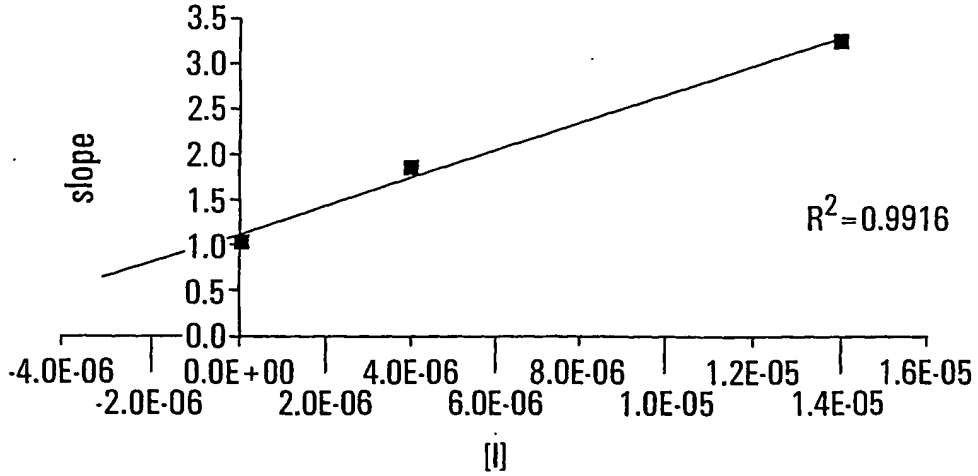


FIG. 21B

Ki
BuChE + BuTCh + Ethylene diamine urea derivative of Phenothiazine (#2)



$K_i = 7.13 \times 10^{-6}$ M

FIG. 21C

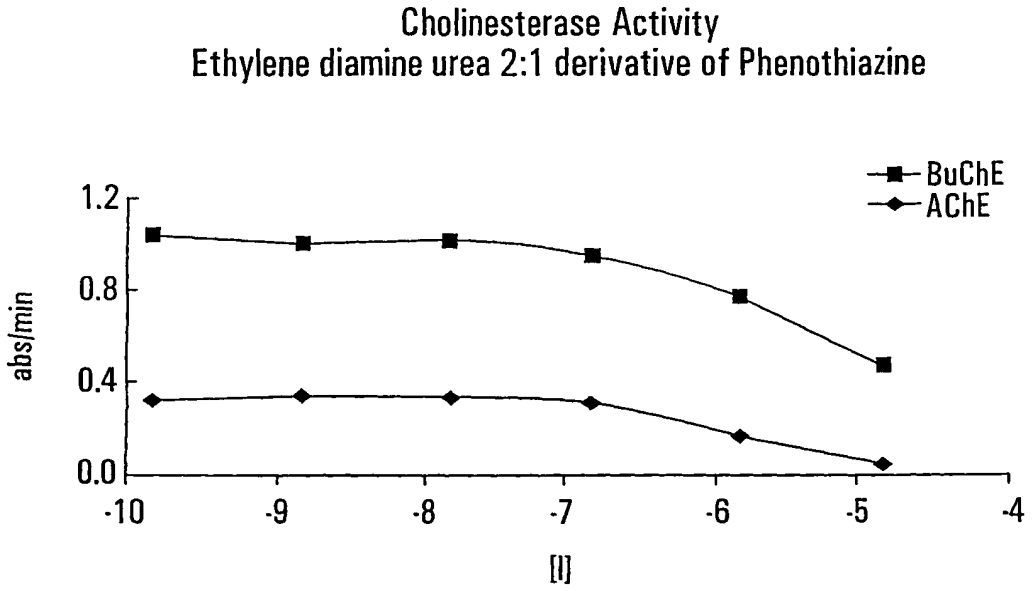


FIG. 22A

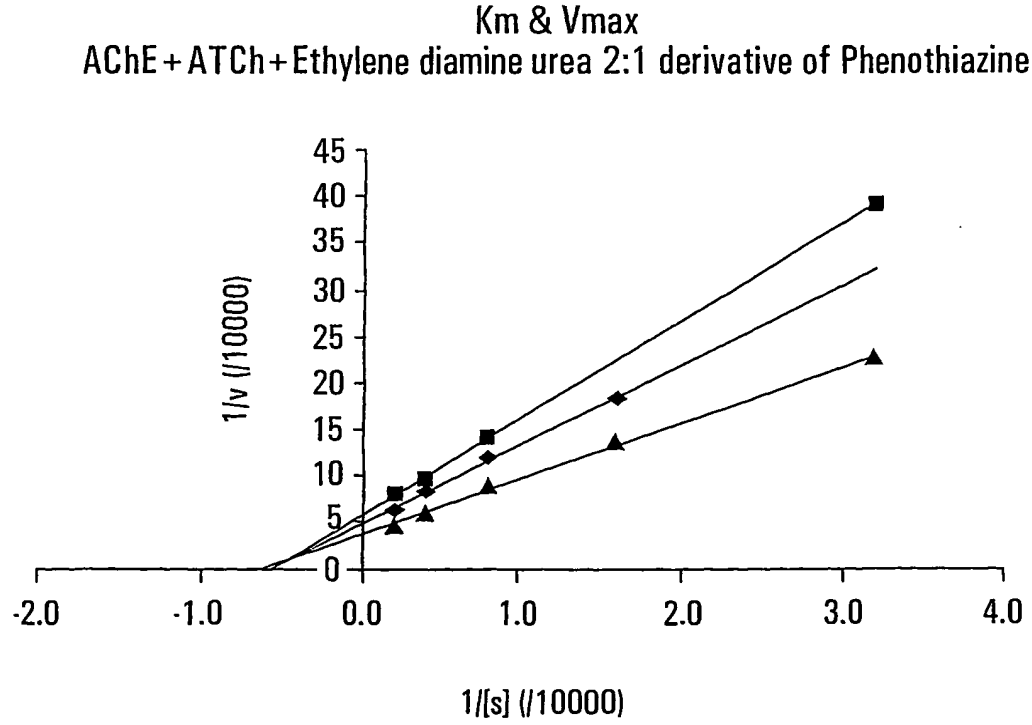


FIG. 22B

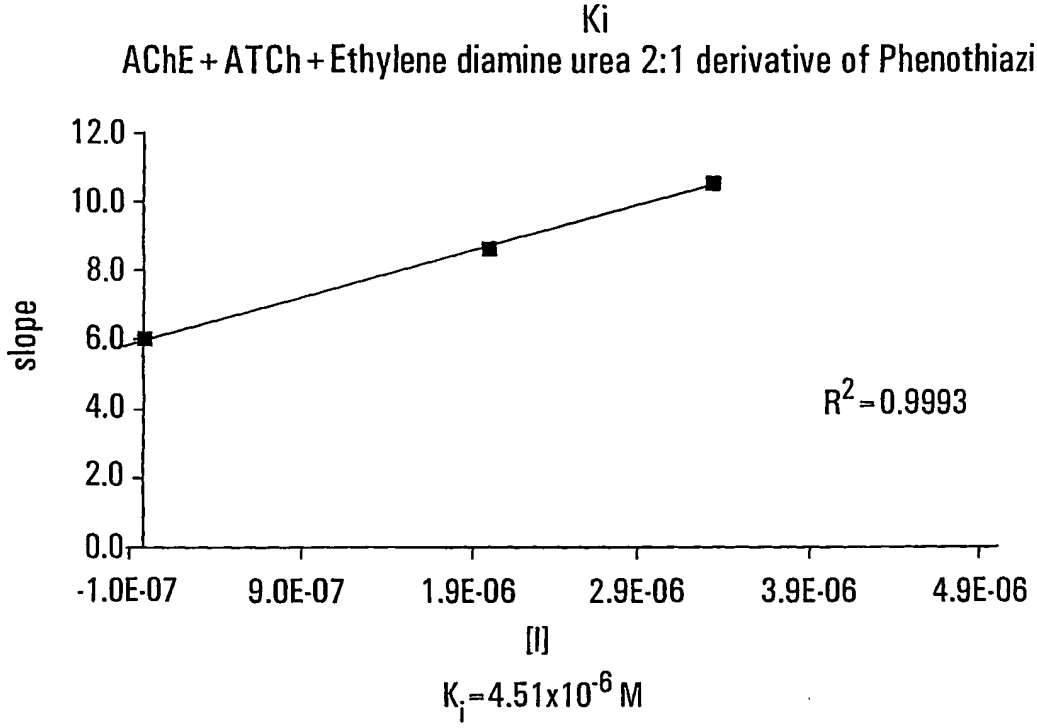


FIG. 22C

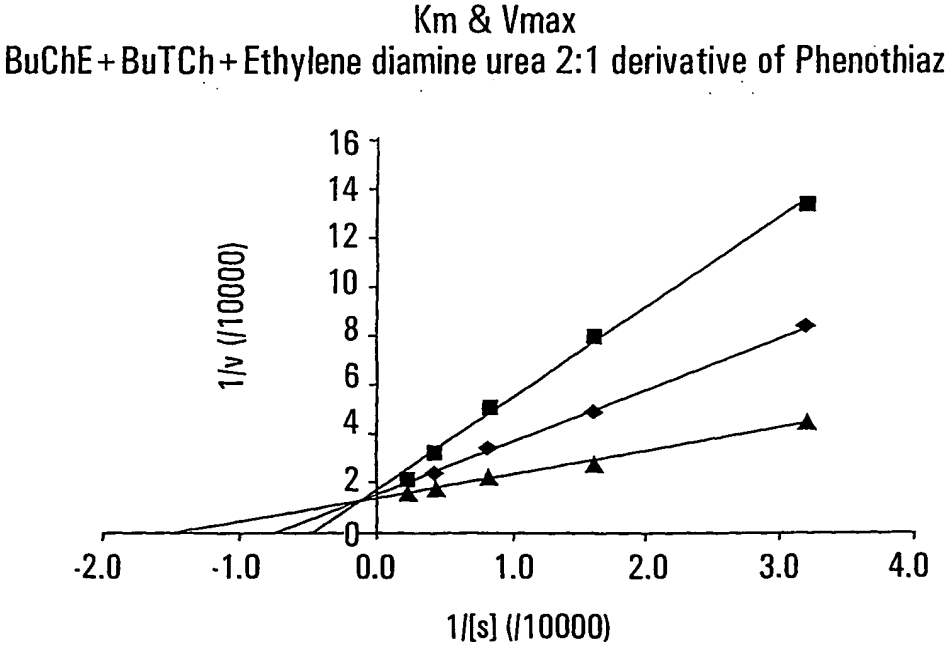


FIG. 22D

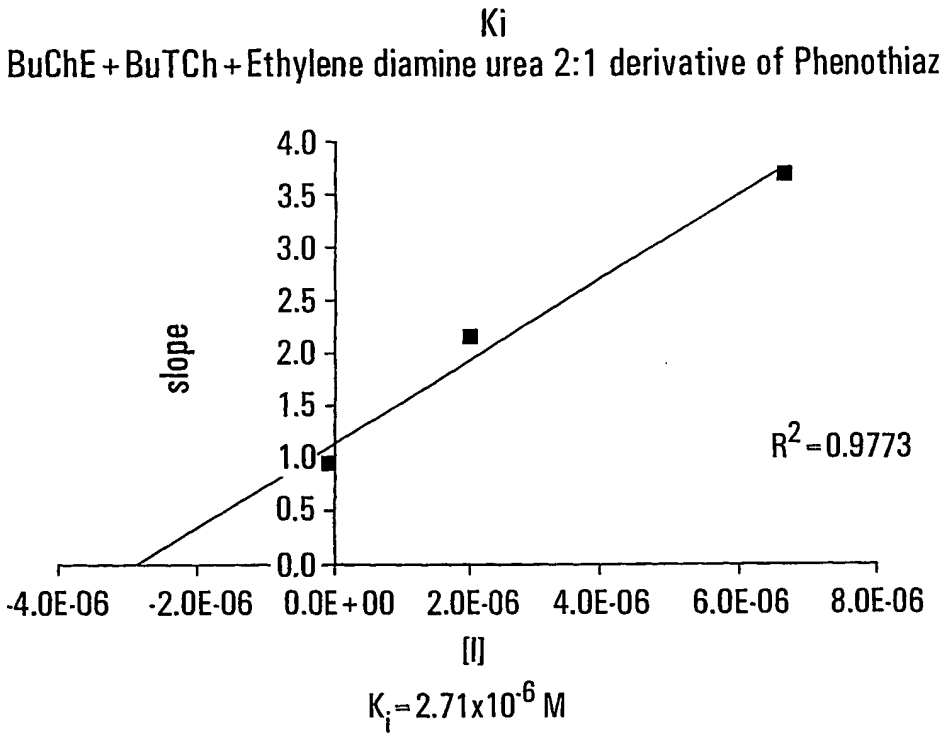


FIG. 22E

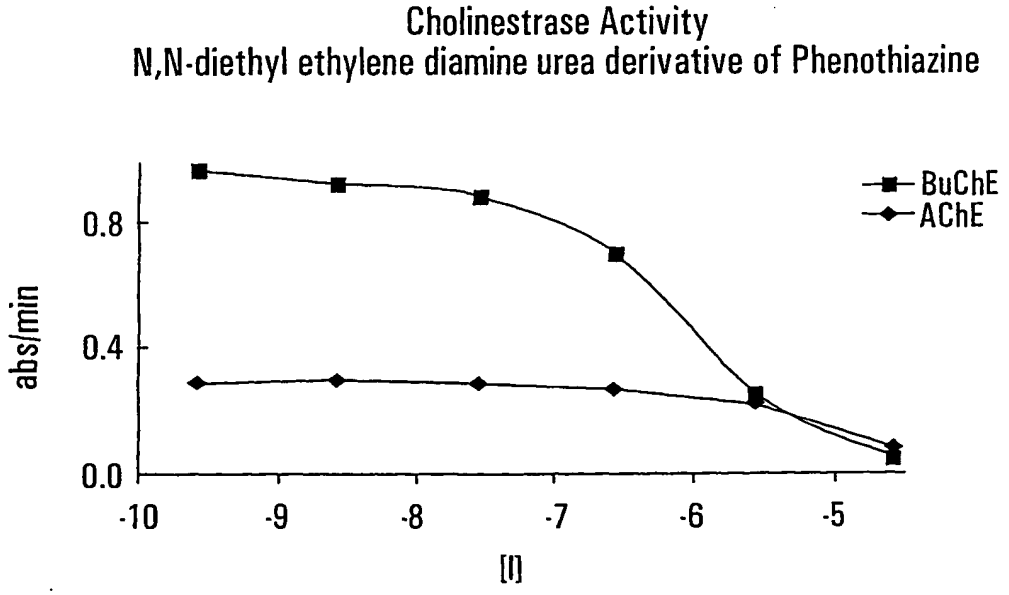


FIG. 23A

Km & Vmax
AChE + ATCh + N,N-diethyl ethylene diamine urea derivative of Phenothiazine

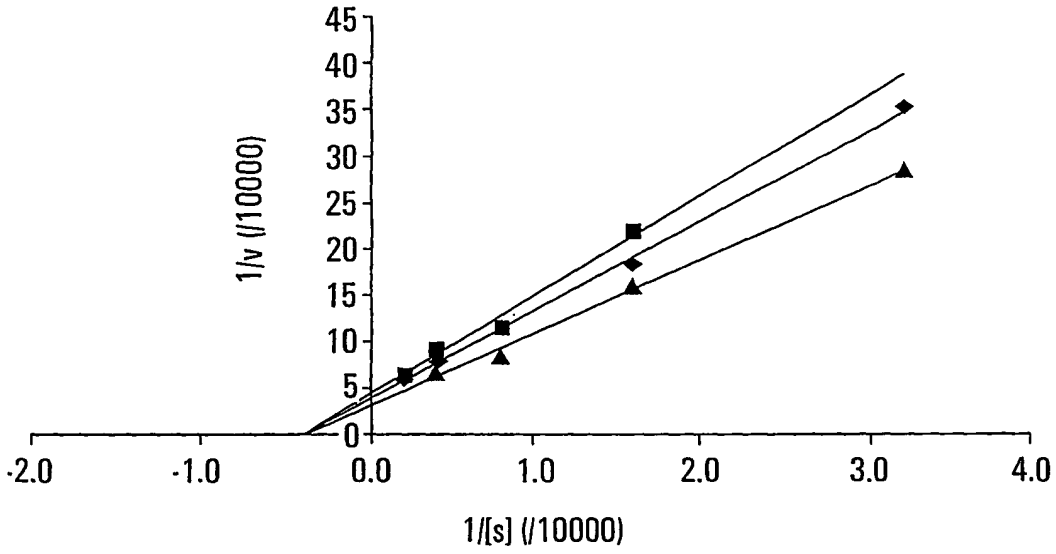
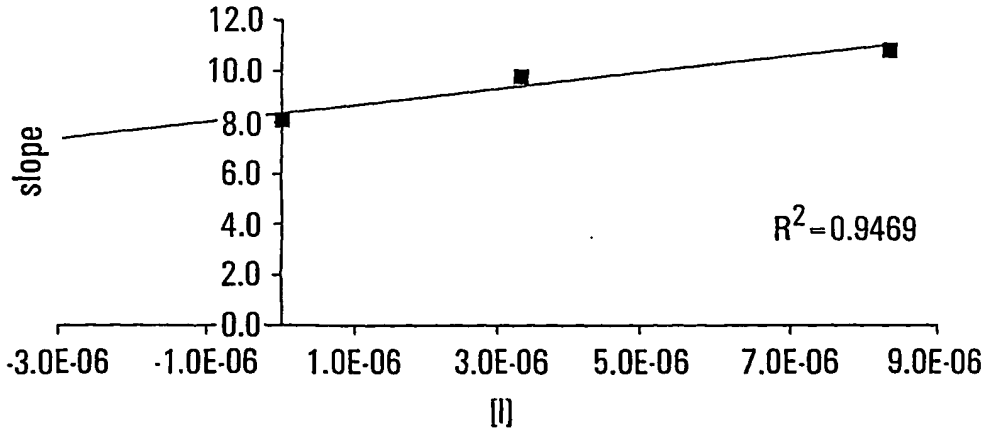


FIG. 23B

Ki
AChE + ATCh + N,N-diethyl ethylene diamine urea derivative of Phenothiazine



$K_i = 2.41 \times 10^{-5}$ M

FIG. 23C

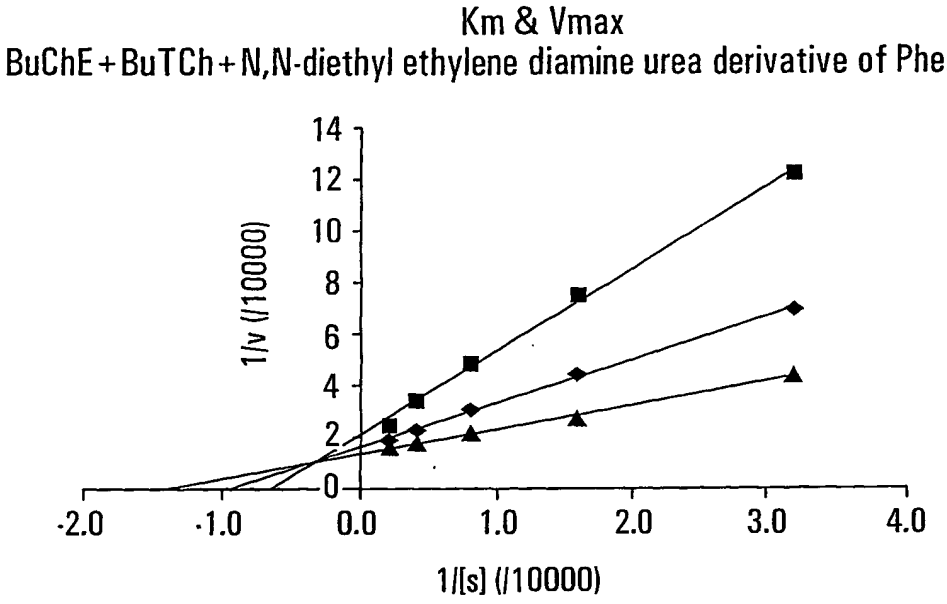


FIG. 23D

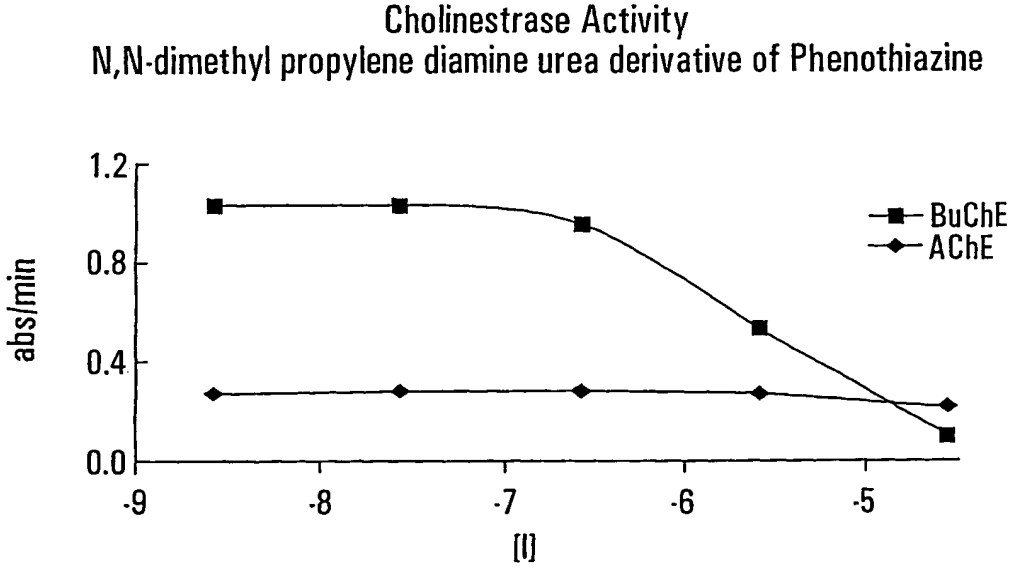


FIG. 24A

Km & Vmax
BuChE+BuTCh +N,N-dimethyl propylene diamine urea derivative of Phenothiazine

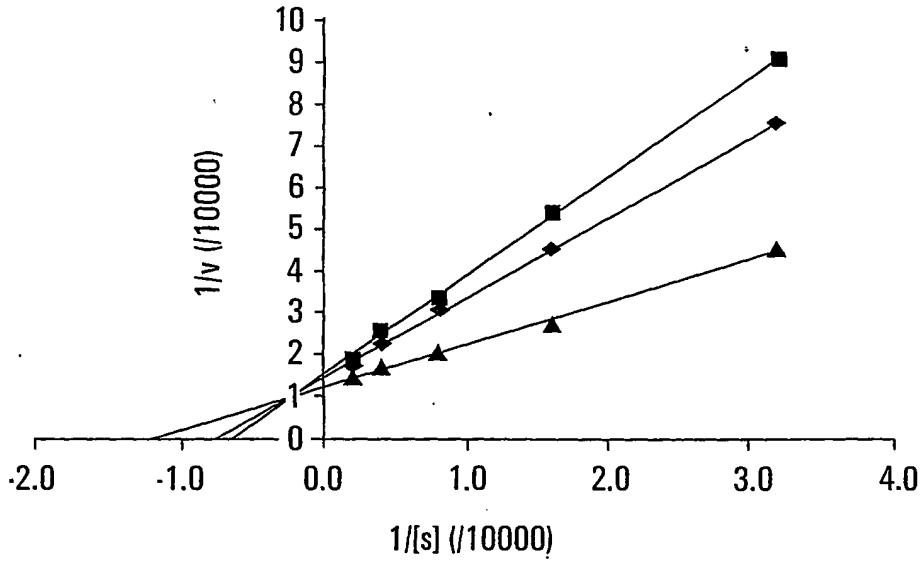
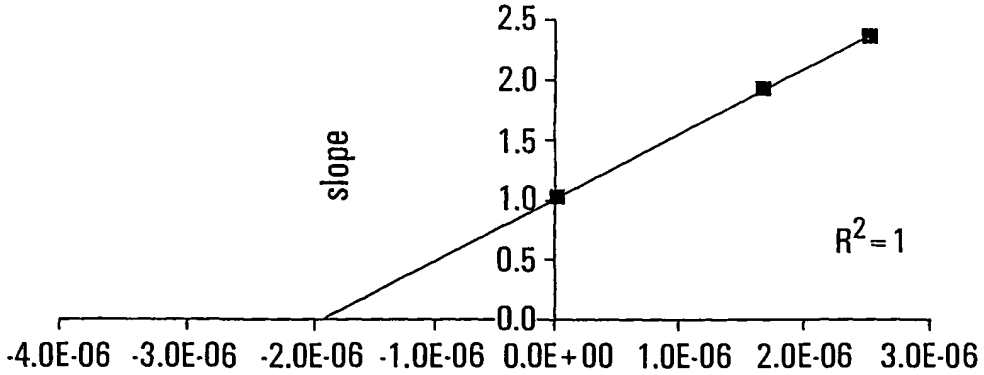


FIG. 24B

Ki
BuChE+BuTCh+N,N-dimethyl propylene diamine urea derivative of Phenothiazine



$K_i = 1.90 \times 10^{-6}$ M

FIG. 24C

Km & Vmax

AChE + ATCh + N,N-dimethyl propylene diamine urea derivative of Phenothiazine

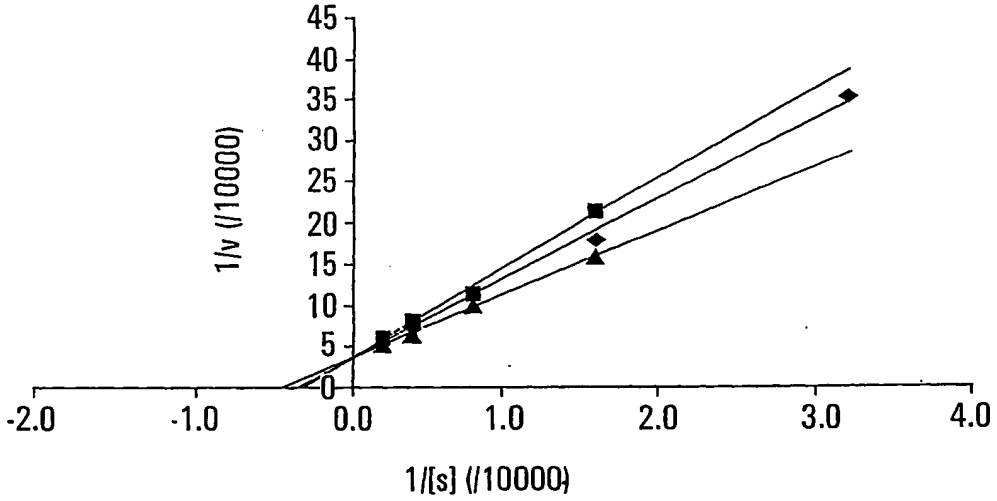


FIG. 24D

Ki

AChE + ATCh + N,N-dimethyl propylene diamine urea derivative of Phenothiazine

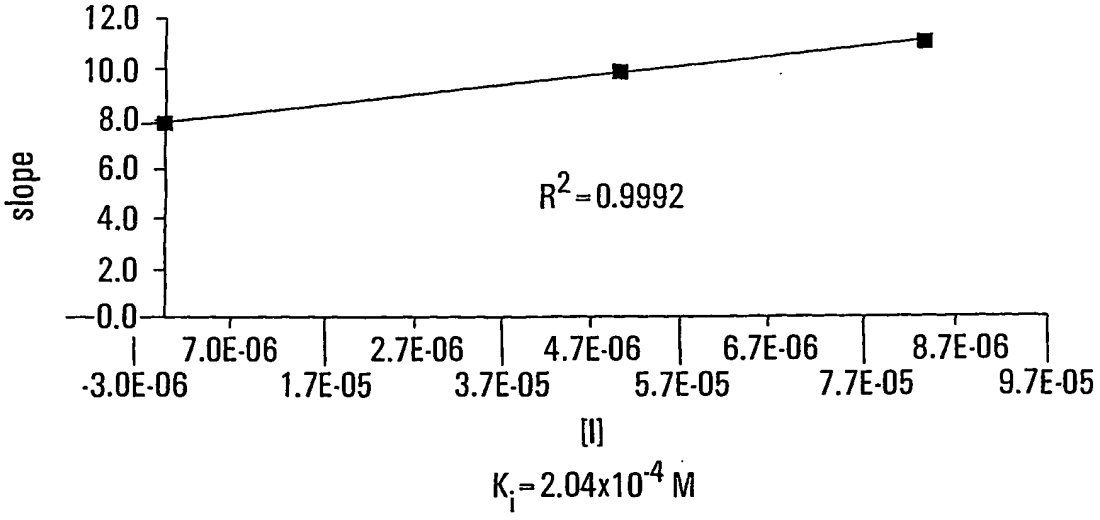


FIG. 24E

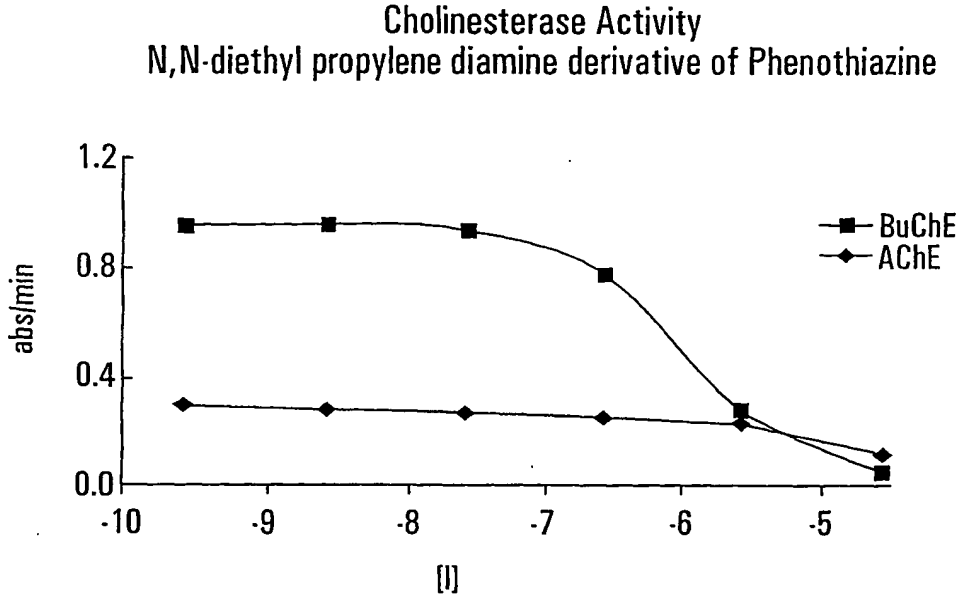


FIG. 25A

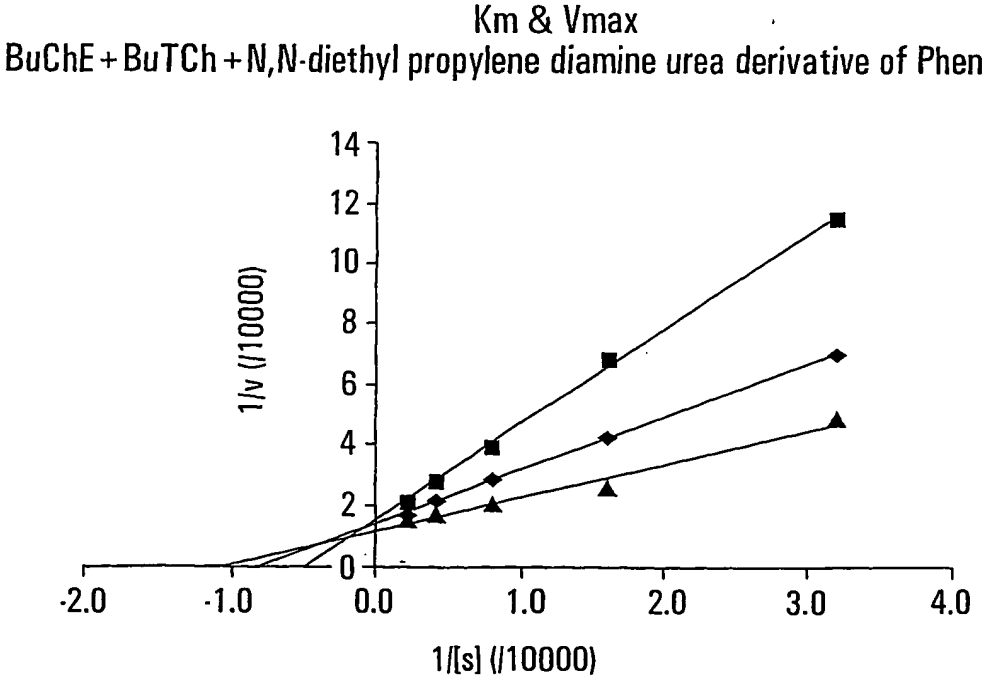
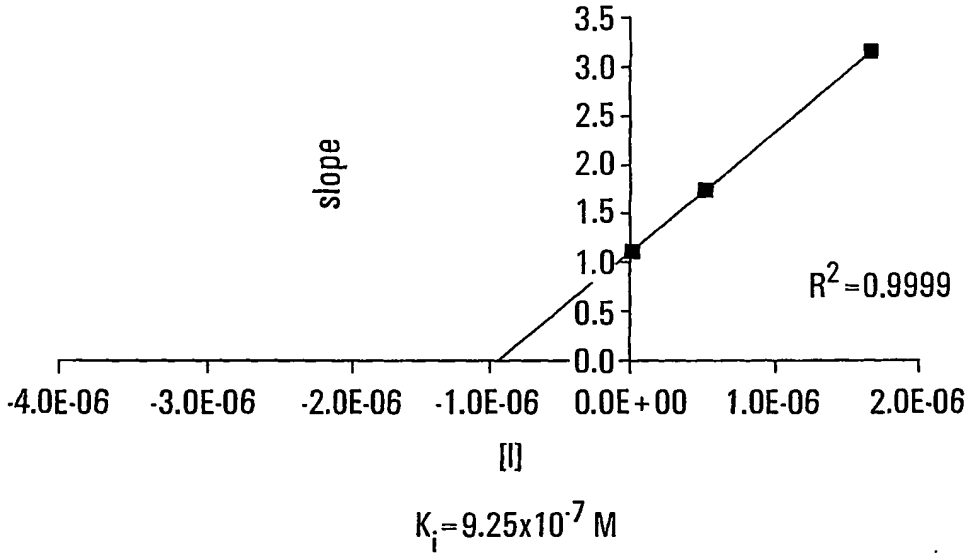


FIG. 25B

Ki

BuChE + BuTCh + N,N-diethyl propylene diamine urea derivative of Phenothiazine



$K_i = 9.25 \times 10^{-7}$ M

FIG. 25C

Km & Vmax

AChE + ATCh + N,N-diethyl propylene diamine urea derivative of Phenothiazine

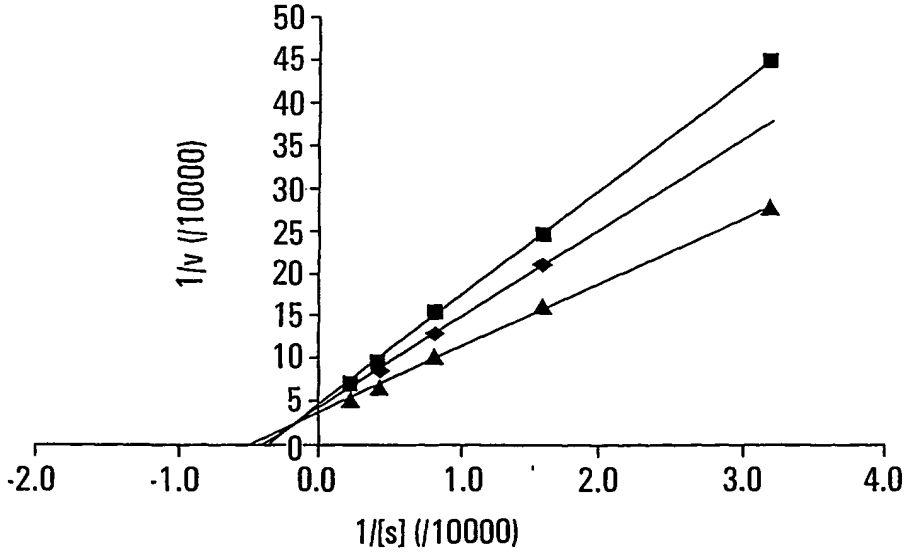


FIG. 25D

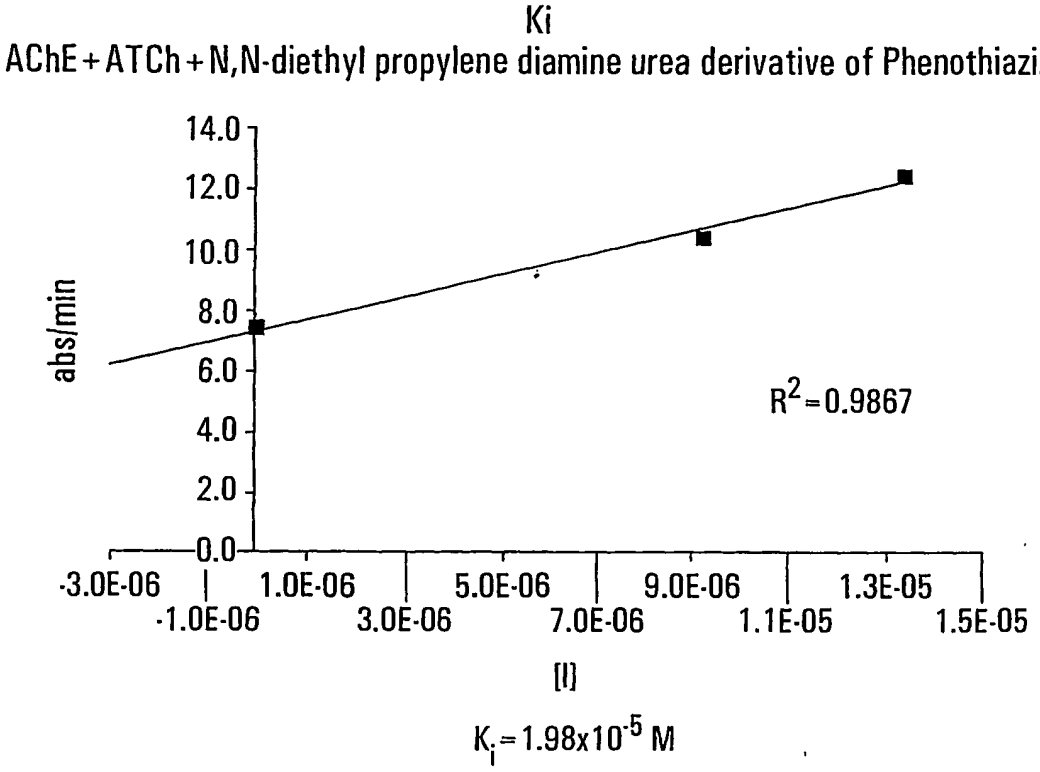


FIG. 25E

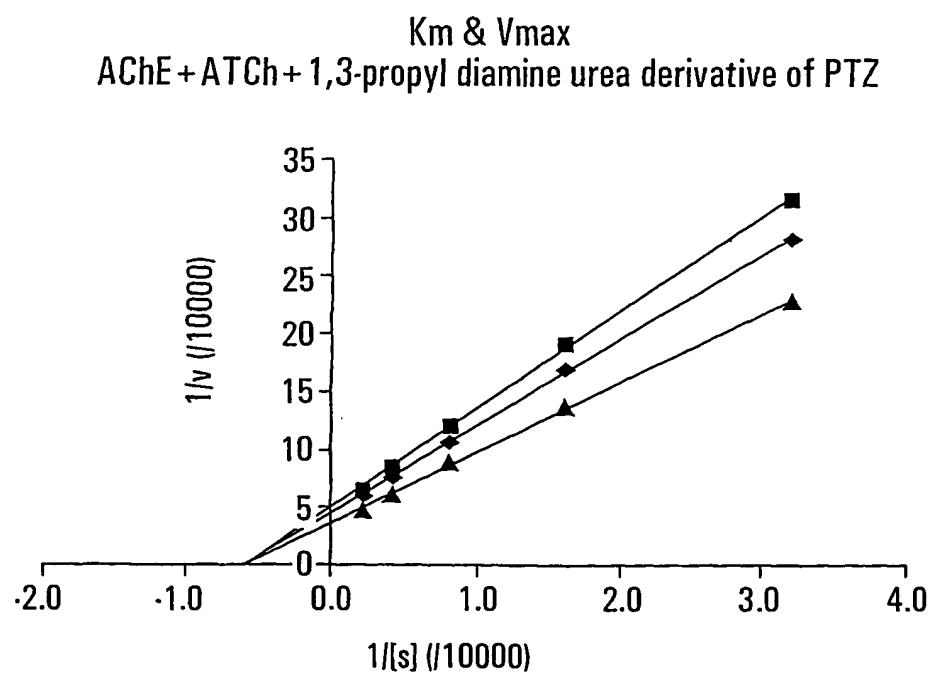


FIG. 26A

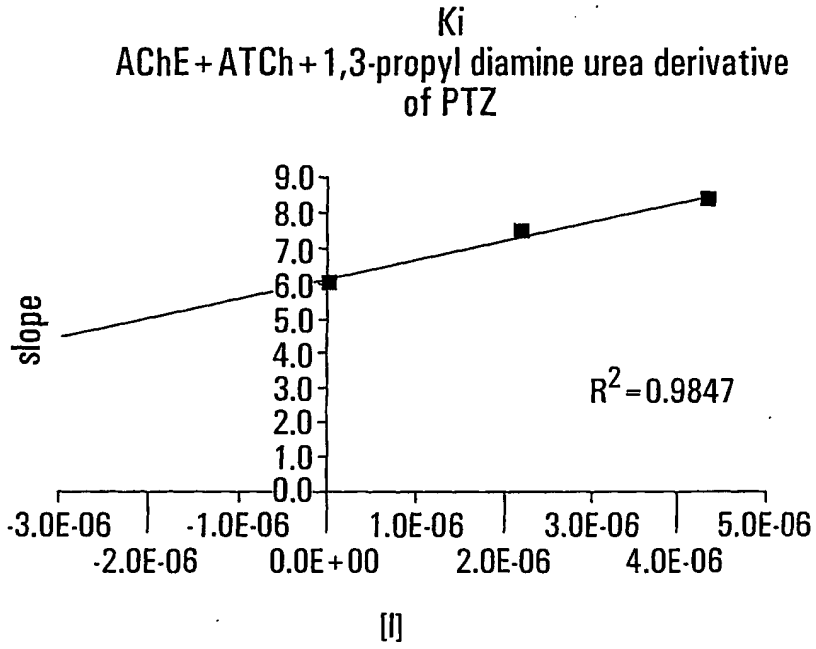

Ki
AChE + ATCh + 1,3-propyl diamine urea derivative of PTZ
slope
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
R^2 = 0.9847
-3.0E-06
-2.0E-06
-1.0E-06
0.0E+00
1.0E-06
2.0E-06
3.0E-06
4.0E-06
5.0E-06
[I]

FIG. 26B

INTERNATIONAL SEARCH REPORT

Inter Application No
PCT/CA 01/00772

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D279/30 A61K31/5415 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 05837 A (BAYER AG ;URBAHNS KLAUS (DE); HEINE HANS GEORG (DE); JUNGE BODO (D) 29 February 1996 (1996-02-29) the whole document	1-46
X	US 4 833 138 A (OLNEY JOHN W) 23 May 1989 (1989-05-23) the whole document	1-46
X	FR 2 303 542 A (FABRE SA PIERRE) 8 October 1976 (1976-10-08) the whole document	1-8,15, 18

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2001	06/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chouly, J

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INTERNATIONAL SEARCH REPORT

Inte Application No
PCT/CA 01/00772

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 96, no. 17, 26 April 1982 (1982-04-26) Columbus, Ohio, US; abstract no. 142872, SHOWA DENKO K.K.: "Antitumor phenothiazine derivatives" XP002175963 abstract & JP 81 166183 A 21 December 1981 (1981-12-21)	1-8,18
X	US 4 122 199 A (COUSSE HENRI ET AL) 24 October 1978 (1978-10-24) the whole document	1-8,15, 18
X	EP 0 138 481 A (MERCK FROSST CANADA INC) 24 April 1985 (1985-04-24) claims	1,18
X	CH 653 675 A (CIBA GEIGY AG) 15 January 1986 (1986-01-15) the whole document	1,18
A	FR 1 192 168 A (M. DELALANDE) 23 October 1959 (1959-10-23) the whole document	1,18
A	GB 1 071 815 A (BOOTS PURE DRUG CO LTD) 14 June 1967 (1967-06-14) the whole document	1,18

INTERNATIONAL SEARCH REPORT

Inte Application No
PCT/CA 01/00772

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9605837 A	29-02-1996	DE 4430091 A	29-02-1996
		AU 3345395 A	14-03-1996
		CA 2198256 A	29-02-1996
		EP 0777481 A	11-06-1997
		IL 115027 A	31-01-2000
		JP 10504820 T	12-05-1998
		TW 408117 B	11-10-2000
		US 5861394 A	19-01-1999
		ZA 9507113 A	15-04-1996
US 4833138 A	23-05-1989	NONE	
FR 2303542 A	08-10-1976	NONE	
JP 81166183 A		NONE	
US 4122199 A	24-10-1978	FR 2361883 A	17-03-1978
		BE 844664 A	16-11-1976
		CH 611612 A	15-06-1979
		DE 2632200 A	20-10-1977
		ES 450299 A	01-12-1977
		GB 1554912 A	31-10-1979
		US 4192883 A	11-03-1980
EP 0138481 A	24-04-1985	CA 1272722 A	14-08-1990
		DE 3484745 D	01-08-1991
		DK 475684 A	06-04-1985
		ES 536525 D	01-04-1988
		ES 8802144 A	16-06-1988
		JP 60155165 A	15-08-1985
		US 4845083 A	04-07-1989
		US 4666907 A	19-05-1987
CH 653675 A	15-01-1986	NONE	
FR 1192168 A	23-10-1959	NONE	
GB 1071815 A	14-06-1967	NONE	